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REVIEW ARTICLE

What do mouse models of muscular dystrophy tell us about the DAPC and its components?

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SUMMARY

There are over 30 mouse models with mutations or inactivations in the dystrophin-associated protein complex. This complex is thought to play a crucial role in the functioning of muscle, as both a shock absorber and signalling centre, although its role in the pathogenesis of muscular dystrophy is not fully understood. The first mouse model of muscular dystrophy to be identified with a mutation in a component of the dystrophin-associated complex (dystrophin) was the mdx mouse in 1984. Here, we evaluate the key characteristics of the mdx in comparison with other mouse mutants with inactivations in DAPC components, along with key modifiers of the disease phenotype. By discussing the differences between the individual phenotypes, we show that the functioning of the DAPC and consequently its role in the pathogenesis is more complicated than perhaps currently appreciated.

Keywords
dystroglycan, dystrophin, mdx, mouse models, muscular dystrophy

Introduction

Muscular dystrophy is a family of inherited conditions, with a spectrum of phenotypes, caused by mutations in a number of genes associated with muscle development, structure, maintenance, function or repair. A wide variety and number of different mouse models have been generated (over 55 strains are listed on the Jackson Laboratory web page – http://jaxmice.jax.org/neurobiology/muscular-dystrophy.html) to investigate the phenotype of these human conditions and test potential therapeutic strategies. Consequently, a review of muscular dystrophy mouse models is a huge topic, shown by over 8000 results from a Web of Science search for muscular dystrophy mouse models, so we will restrict ourselves to a particular subset of muscular dystrophy mouse models. It is currently thought in some of these conditions that muscle fibres degenerate because they are unable to withstand the mechanical forces of contraction, due to compromised functioning of the dystrophin-associated protein complex (DAPC). The DAPC (shown as a cartoon schematic in Figure 1) is a large multimeric protein complex that links the intracellular actin filaments to the extracellular basement membrane (Michele & Campbell 2003) and is considered to have roles including acting as shock absorber in muscle. Therefore, we have chosen to focus on mouse models where the phenotype is caused by compromised functioning of the DAPC, through mutation and/or inactivation in DAPC components, such as dystrophin, α-dystrobrevin, dystroglycan, the sarcoglycan complex and sarcospan, or through proteins that post-translationally modify DAPC components, such as POMT1, POMT2, POMGnT1, FKRP, fukutin and LARGE, enzymes that are involved in α-dystroglycan glycosylation. At the time of writing, this is still 43 mouse models, although we have

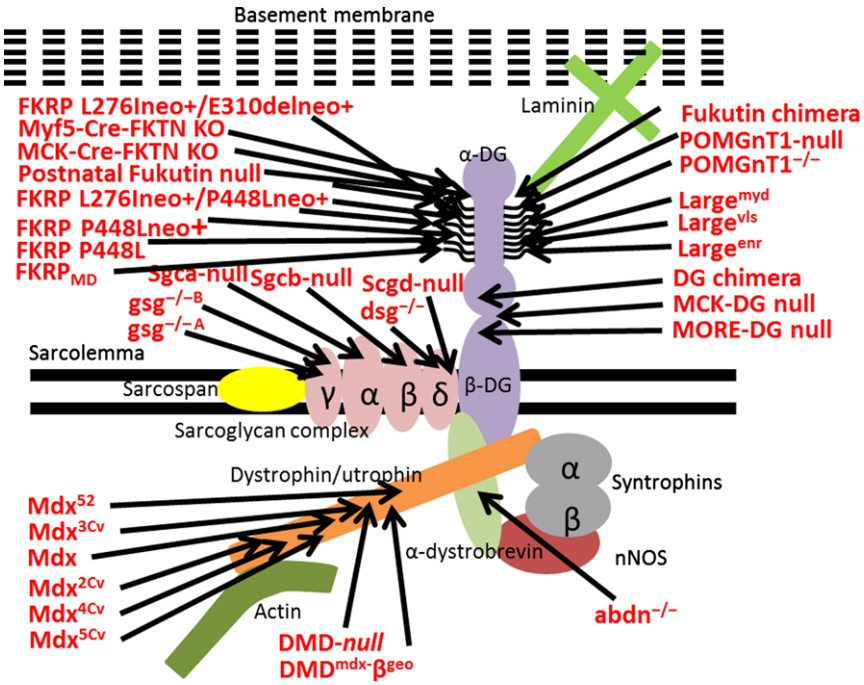

Figure 1 Schematic of dystrophin-associated protein complex (DAPC). Cartoon schematic showing the muscle dystrophin-associated protein complex (DAPC), composed of dystroglycan (both α- and β-subunits are shown), sarcospan, sarcoglycan complex, dystrophin or utrophin, α-dystrobrevin, the syntrophins, actin (which binds to dystrophin) and neuronal nitrogen oxide synthase (nNOS), which binds to α-dystrobrevin and the syntrophins. These different components are labelled in the diagram and are shown in different colours. We will not be describing the structure and function of the different DAPC components, and for more information, the reader is directed to Michele and Campbell (2003). The mouse models mentioned in this review are shown in the figure, with an arrow indicating the affected protein component.

excluded a number of mutants – Dag-1 null (Williamson *et al.* 1997), FKRP^{KD} mouse (Ackroyd *et al.* 2009), FKRP E310delneo+ (Chan *et al.* 2010), FKRP P448Lneo+/E310delneo+ compound heterozygotes (Blaeser *et al.* 2013), fukutin null (Kurahashi *et al.* 2005), POMT1$^{-/-}$ (Willer *et al.* 2004) and POMT2 null (Hu *et al.* 2011) – as they do not exhibit any obvious muscle abnormality because they die either prenatally or in the early postnatal period. We have also chosen to exclude those that do not carry a germline mutation, for example where postnatal knock-down is achieved through shRNA, as well as the fukutin Hp^{-} (Kanagawa *et al.* 2009), Spsn-deficient (Lebakken *et al.* 2000), Scge-null (Lancioni *et al.* 2011) and α-syn$^{-/-}$ mice (Adams *et al.* 2000), as these do not develop a discernable dystrophic phenotype. Furthermore, we have also chosen to exclude mouse models with mutations or inactivations in neuronal nitric oxide synthase (nNOS) (Huang *et al.* 1993). The DAPC is thought to fulfil two roles in muscle fibres, a mechanical role, as a shock absorber to minimize contraction-induced damage, and a biochemical role, acting as a signalling centre. All components of the DAPC are involved in both mechanical and biochemical roles, with the exception of nNOS, which is mainly involved with signalling pathways. Whilst these signalling pathways are important for muscle development, maintenance and repair, we feel that encompassing major discussions on these signalling pathways would be outside the scope of this review. Recently, a review has been published on the various signalling roles of the DAPC (Constantin 2014), and we direct the reader to this publication for more details on this topic.

All 32 models included here carrying mutations or inactivations in the components of the DAPC evaluated in the review are listed in Table 1. To make the text easier to read, rather than listing the citations after each individual point, the main references for each animal model have been included in Table 1. In this review, first we provide a short summary of the most well-known mouse mutants with inactivation in a DAPC component – the dystrophin-deficient mdx models – before comparing these to the other models with mutations/inactivations in the DAPC, exploring what any similarities or differences mean for the functioning of the DAPC. It is important to note here that if no comment is made on a parameter in a certain model, then it simply means that no comment has been made on this parameter in the literature. In this way, we hope to illustrate that the DAPC is an extremely complex structure that is not as well understood as we like to think.

Both γ-sarcoglycan mouse mutants are referred to in their publications as gsg$^{-/-}$, so the letters A and B refer to the models so the two models can be differentiated in the text. Additionally, in the sections on mdx mouse models, we have specified which have lost the Dp40 isoform based on information from Tozawa *et al.* (2012).

Dystrophin-deficient models

The original dystrophin-deficient model, the mdx mouse, was discovered by chance in 1984 as it had increased levels of muscle creatinine kinase (MCK) and pyruvate kinase in the serum, along with histological indicators of skeletal muscle degeneration and regeneration (Carnwath & Shotton 1987; Coulton *et al.* 1988). It was then found to lack the protein dystrophin that is missing in human Duchenne muscular dystrophy patients (Hoffman *et al.* 1987) and to have a point mutation in exon 23 of the dystrophin gene (Sicinski *et al.* 1989). In the 30 years since its discovery, the mdx

Table 1 Mouse muscular dystrophy models with inactivations or mutations in DAPC components

Mouse model name and mutated gene	DAPC Component	Nature of mouse model	References	Model of human disease
Mdx *Dystrophin*	Dystrophin	Spontaneous mutation exon 23 – loss of Dp427 isoform	Bulfield *et al.* (1984), Barton (2006)	Duchenne muscular dystrophy (DMD)
Mdx^{2Cv} *Dystrophin*	Dystrophin	Mutation intron 42 - loss of Dp427 and Dp260 isoforms	Chapman *et al.* (1989), Im *et al.* (1996)	DMD
Mdx^{3Cv} *Dystrophin*	Dystrophin	Mutation exon 65 – loss of Dp427, Dp260, Dp140, Dp116, Dp40 and Dp71 isoforms	Chapman *et al.* (1989), Cox *et al.* (1993b)	DMD
Mdx^{4Cv} *Dystrophin*	Dystrophin	Mutation exon 53 – loss of Dp427, Dp260 and Dp140 isoforms	Chapman *et al.* (1989), Im *et al.* (1996)	DMD
Mdx^{5Cv} *Dystrophin*	Dystrophin	Mutation exon 10 – loss of Dp427 isoform	Im *et al.* (1996)	DMD
Mdx^{52} *Dystrophin*	Dystrophin	Mutation exon 52 – loss of Dp427, Dp260 and Dp140 isoforms	Araki *et al.* (1997)	DMD
$DMD^{mdx-\beta geo}$ *Dystrophin*	Dystrophin	Mutation exon 63 – loss of all dystrophin isoforms	Wertz and Fuchtbauer (1998)	DMD
DMD-*null* *Dystrophin*	Dystrophin	Global knockout of dystrophin isoforms	Kudoh *et al.* (2005)	DMD
MCK-DG null *Dag1 – Dystroglycan*	Dystroglycan	Conditional knockout under muscle creatinine kinase (MCK) promoter	Cohn *et al.* (2002)	N/A
MORE-DG null *Dag1*	Dystroglycan	Conditional knockout – everywhere except Reichert's membrane	Satz *et al.* (2008)	N/A
DG chimera *Dag1 – Dystroglycan1*	Dystroglycan	Dystroglycan chimera	Cote *et al.* (1999)	N/A
POMGnT1-null *POMGnT1 – Protein-O-mannose β-1,2-N-acetylglucosaminyltransferase*	Dystroglycan glycosylation	Global knockout of POMGnT1	Liu *et al.* (2006)	Walker–Warburg syndrome (WWS), muscle–eye–brain (MEB) disease, limb girdle muscular dystrophy type 2M (LGMD2O)
$POMGnT1^{-/-}$ *POMGnT1*	Dystroglycan glycosylation	Global knockout POMGnT1	Miyagoe-Suzuki *et al.* (2009)	WWS, MEB disease, LGMD2O
$FKRP_{MD}$ *Fkrp – fukutin-related protein*	Dystroglycan glycosylation	Knock-down in all cells except those expressing Sox1	Whitmore *et al.* (2014)	WWS, MEB disease, congenital muscular dystrophy type 1C (MDC1C), LGMD 2I
FKRP P448Lneo+ *Fkrp*	Dystroglycan glycosylation	Global knock-down in expression	Chan *et al.* (2010)	WWS, MEB disease, MDC1C, LGMD2I
FKRP P448L *Fkrp*	Dystroglycan glycosylation	Global point mutation	Blaeser *et al.* (2013)	WWS, MEB disease, MDC1C, LGMD2I
FKRP L276Ineo+/P448Lneo+ *Fkrp – fukutin-related protein*	Dystroglycan glycosylation	Global point mutation + reduction in expression	Blaeser *et al.* (2013)	WWS, MEB disease, MDC1C, LGMD2I
FKRP L276Ineo+/E310delneo+*Fkrp*	Dystroglycan glycosylation	Global point mutation + reduction in expression	Blaeser *et al.* (2013)	WWS, MEB disease, MDC1C, LGMD2I
$Large^{myd}$ *Large – Like-acetylglucosaminyltransferase*	Dystroglycan glycosylation	Global knockout *Large*	Lane *et al.* (1976), Mathews *et al.* (1995) Grewal *et al.* (2005)	WWS, MEB disease, MDC1D
$Large^{enr}$ *Large*	Dystroglycan glycosylation	Global knockout *Large*	Kelly *et al.* (1994) Levedakou *et al.* (2005)	WWS, MEB disease, MDC1D
$Large^{vls}$ *Large*	Dystroglycan glycosylation	Global knockout *Large*	Lee *et al.* (2005)	WWS, MEB disease, MDC1D
Myf5-Cre-FKTN-KO *Fktn – fukutin*	Dystroglycan glycosylation	Conditional knockout under Myf5 promoter	Beedle *et al.* (2012)	WWS, MEB disease, FCMD, LGMD2M

Table 1. Continued

Mouse model name and mutated gene	DAPC Component	Nature of mouse model	References	Model of human disease
MCK-Cre-FKTN-KO *Fktn*	Dystroglycan glycosylation	Conditional knockout of under MCK promoter	Beedle *et al.* (2012)	WWS, MEB disease, FCMD, LGMD2M
Postnatal fukutin null *Fktn – fukutin*	Dystroglycan glycosylation	Global knockout of fukutin from 6 weeks	Beedle *et al.* (2012)	WWS, MEB disease, FCMD, LGMD2M
Fukutin chimera *Fktn – fukutin*	Dystroglycan glycosylation	Fukutin chimera	Takeda *et al.* (2003)	WWS, MEB disease, Fukuyama congenital muscular dystrophy (FCMD), LGMD2M
Sgca-null *Sgca – α-sarcoglycan*	α-Sarcoglycan	Global knockout	Duclos *et al.* (1998), Consolino *et al.* (2005), Jakubiec-Puka *et al.* (2005), Patel *et al.* (2003)	LGMD2D
Sgcb-null *Sgcb – β-sarcoglycan*	β-Sarcoglycan	Global knockout	Durbeej *et al.* (2000), Andersson *et al.* (2012)	LGMD2E
$gsg^{-/-A}$ *Sgcg – γ-sarcoglycan*	γ-Sarcoglycan	Global knockout	Hack *et al.* (1998, 1999), Barton (2006)	LGMD2C
$gsg^{-/-B}$ *Sgcg*	γ-Sarcoglycan	Global knockout	Sasaoka *et al.* (2003)	LGMD2C
$dsg^{-/-}$ *Sgcd – δ-sarcoglycan*	δ-Sarcoglycan	Global knockout	Hack *et al.* (2000)	LGMD2F
Scgd-null *Sgcd*	δ-Sarcoglycan	Global knockout	Coral-Vazquez *et al.* (1999), Wansapura *et al.* (2011)	LGMD2F
$abdn^{-/-}$ *abdn – α-dystrobrevin*	α-Dystrobrevin	Global knockout of α-dystrobrevin splice isoforms with exon 3	Grady *et al.* (1999), Bunnell *et al.* (2008)	Left ventricular non-compaction 1 (LVNC1)

This table lists all the mouse models mentioned in this review by name, identifying which part of the DAPC is affected and describing the nature of the model. For ease of reading, we have left the frequently cited references out of the text, so this table also contains those references used to gather the majority of the information about each mouse model.

mouse has been used as a preclinical model of dystrophin restoration mediated by means such as stem or precursor cells (Partridge *et al.* 1989), plasmids, viral vectors [reviewed (Konieczny *et al.* 2013)] stop codon read-through and antisense oligonucleotide-mediated exon skipping. Several other mouse models for dystrophin deficiency exist (Table 1), which have mutations in different parts of *dystrophin* – the mutation in mdx^{2cv} is in intron 42, mdx^{4cv} in exon 53, mdx^{5cv} in exon 10, mdx^{3cv} in exon 65, mdx^{52} in exon 52 and $DMD^{mdx\text{-}\beta geo}$ in exon 63. However, with the exception of the $DMD^{mdx\text{-}\beta geo}$ mouse, all of these models express shorter dystrophin isoforms (Chamberlain *et al.* 1993) [reviewed (Willmann *et al.* 2009)] and have naturally occurring revertant (dystrophin-expressing) fibres (Hoffman *et al.* 1990; Lu *et al.* 2000), although mdx^{4cv}, mdx^{5cv} and mdx^{52} have fewer reverent fibres than mdx mice (Danko *et al.* 1992; Echigoya *et al.* 2013). As all of these dystrophin-deficient mouse models have a similar, although not always identical, pathology (Beastrom *et al.* 2011), we will discuss, unless otherwise indicated, the most widely used model, the original mdx.

Age of onset

These mouse models of muscular dystrophy have a range in the reported age of onset, varying from birth for the MORE-DG null to 10 days for mdx mice (Torres & Duchen 1987); 2 weeks for the $Large^{myd}$, Scgb-null, Scga-null, $Large^{enr}$ and Sgcd-null models; 12 weeks in the MCK-Cre-FKTN-KO; and finally 14 weeks in the postnatal fukutin null, although the most common age of onset for these models is 4–8 weeks of age (fukutin chimera, Myf5-Cre-FKTN-KO, FKRP P448L-neo+, $FKRP_{MD}$, $Large^{enr}$, $Large^{vls}$, MCK-DG null, POMGnT1-null, $dsg^{-/-}$, $gsg^{-/-A}$, $abdn^{-/-}$). This wide range in the age of onset can most likely be attributed to a combination of two reasons – first the presence of central nervous system defects in some of these models, which may exacerbate the general phenotype or make it more pronounced, so symptoms are evident at an earlier age. Many studies have shown that the DAPC is involved in cortical development and function [reviewed in (Waite *et al.* 2009)], and this can be seen in these animal models with inactivations in the DAPC as many display central nervous system defects, including the MORE-DG null, $Large^{myd}$, $Large^{vls}$, $Large^{enr}$ and the mdx mouse. However, the different components of the DAPC have varying roles in the central nervous system (Waite *et al.* 2009) with α-dystroglycan shown to be important for neuronal migration and dystrophin crucial for postsynaptic clustering of GABAergic receptors (Brunig *et al.* 2002) and functioning of cholinergic synapses (Parames *et al.* 2014). This is reflected in the different cortical phenotypes of the mouse models, with

alterations in α-dystroglycan expression or structure (MORE-DG, Large^{myd}) more severe than those seen in the mdx. Second, some models included in this review have tissue-specific or induced gene loss (to overcome the lethality of a complete null phenotype), for example the postnatal fukutin null, which in some cases results in the later onset of the phenotype.

In combination, these data suggest that the age of onset does not vary depending on which specific component of the DAPC is lost, but that the characteristics of each individual mouse model, for example global knockout or tissue-specific knockout, determine the age of symptom onset.

Pathology

Histological evidence of skeletal muscle degeneration and regeneration, typified by centrally nucleated muscle fibres and a variation in fibre size, was identified in all models of muscular dystrophy discussed here, with the exception of POMGnT1$^{-/-}$ where neither feature was identified. Similarly to the mdx, inflammatory infiltrates were identified in the DG chimera, adbn$^{-/-}$, gsg^{-B}, Large^{myd}, FKRP$_{MD}$ and fukutin chimera, but not the Large^{enr}, POMGnT1$^{-/-}$, postnatal fukutin null, Myf5-Cre-Fktn-KO or MCK-Cre-Fktn-KO. Although these inflammatory infiltrates are generally considered to be macrophages, which are important for clearing debris from necrotic or degenerating muscle fibres, other cell types including T cells, mast cells, eosinophils and neutrophils have been identified in dystrophic muscle (Nahirney *et al.* 1997; Cai *et al.* 2000; Whitehead *et al.* 2006). These cells directly contribute to muscle pathology, and in the mdx, it has been shown that altering the behaviour of these cells influences muscle pathology; for example, modulating macrophage or T cell populations reduces pathological features such as fibrosis (Morrison *et al.* 2000; Farini *et al.* 2007; Villalta *et al.* 2009; reviewed in (Evans *et al.* 2009; Mann *et al.* 2011)). Therefore, a lack of inflammatory infiltrate could be interpreted as evidence of muscle pathology of reduced severity. This does not seem to segregate with components of the DAPC – although a loss of dystrophin results in inflammatory infiltrates, a loss of α-dystroglycan glycosylation induces inflammatory infiltrates in some models (FKRP$_{MD}$) but not others (POMGnT1$^{-/-}$). Even mutations in the same gene do not necessarily result in the same pathology, with inflammatory infiltrates documented in the Large^{myd} but not the Large^{enr}. However, all mouse models, with the exception of POMGnT1$^{-/-}$, have centrally nucleated muscle fibres, suggesting that the absence of an inflammatory infiltrate does not prevent degeneration (and regeneration) of muscle fibres.

Mdx mice have a florid phase of skeletal muscle degeneration and regeneration between 3 and 5 weeks of age (Carnwath & Shotton 1987; Coulton *et al.* 1988), with muscle fibre necrosis and regeneration continuing, although less conspicuously, throughout life (Pagel & Partridge 1999; Spurney *et al.* 2009). Similarly, gsg$^{-/-B}$ and FKRP$_{MD}$ mice also have a crisis period, albeit at slightly later onset (7–10 and 12 weeks of age, respectively), when histopathological features such as Evans blue dye uptake and muscle fibre degeneration/regeneration were worsened. In the other models, either no crisis period has been reported, or it is thought that they have ongoing fibre necrosis and degeneration with age, for example Sgca-null and abdn$^{-/-}$ mice. Thus, there does not seem to be segregation of the occurrence of a crisis phase with components of the DAPC, with a loss of a sarcoglycan, albeit different ones, inducing a crisis phase in one model (gsg$^{-/-B}$) and not another (Sgca-null). However, it should be noted that with the exception of the FKRP$_{MD}$, all other models with loss of α-dystroglycan glycosylation do not report a crisis phase. This could be due to differences in the nature of the different dystroglycan models – the FKRP$_{MD}$ has a loss of Fkrp expression in all tissues apart from those expressing Sox1, that is, the central nervous system. This suggests that potentially other cell types or factors could be involved. Despite this, an important point to consider is that a lack of reporting on a crisis phase of disease pathology does not mean that it does not exist; it may be that it has not been explored or identified in these models. For example, if the pathology of the mouse model was only evaluated at one age, then it would be difficult to tell whether a crisis period was present in that mutant at a different time. This would also affect the identification of inflammatory infiltrates, as if these models did have a crisis period, and histological evaluation did not occur during this period, then it might be expected that inflammatory infiltrates would be absent from the muscle.

Furthermore, there is some variation in the occurrence of other histological indicators of muscular dystrophy between the different models, particularly replacement of muscle fibres by adipocytes or fibrotic tissue. Pronounced fat infiltration is observed in Scga-null, Scgb-null, Scgd-null, POMGnT1-null, Large^{myd}, dsg$^{-/-}$, Large^{enr}, gsg$^{-/-A}$, Large^{vls}, gsg$^{-/-B}$, dystroglycan chimera and Myf5-Cre-FKTN-null models, but not the mdx, FKRP$_{MD}$, fukutin chimera, abdn$^{-/-}$, postnatal fukutin null, MCK-Cre-FKTN-KO or the MCK-DG null dystroglycan knockout. Fibrosis was not reported in the FKRP$_{MD}$, POMGnT1-null, POMGnT1$^{-/-}$, abdn$^{-/-}$, postnatal fukutin null or MCK-DG null, but it was observed in other models – mdx, Large^{myd}, gsg$^{-/-A}$, Large^{vls}, Large^{enr}, gsg$^{-/-B}$, fukutin chimera, dystroglycan chimera Scgb-null, FKRP P448Lneo+, Sgca-null, FKRP L276Ineo+/P448Lneo+, Sgcd-null and FKRP L276Ineo+/E310delneo+. Here, there are differences between the different DAPC components – models carrying mutations or inactivations in one of the sarcoglycans have replacement of muscle fibres with fibroadipogenic tissue, but those with perturbed α-dystrobrevin expression do not. The mdx, with loss of dystrophin, has fibrosis, but not replacement of muscle fibres with adipocytes. Dystroglycan is more heterogeneous – loss of dystroglycan results in replacement of muscle tissue with fibroadipogenic tissue, but loss of dystroglycan glycosylation is more variable, some models (Large^{myd}) have

replacement by fibrosis and adipocytes, whereas others ($FKRP_{MD}$) do not.

In combination, these data show that the histopathological features of muscular dystrophy pathology (degree of muscle fibre degeneration, loss and replacement by fibrotic tissue or adipocytes) vary between the different mouse models. These data also show, as in the POMGnT1$^{-/-}$, that if there is not a great deal of muscle fibre loss induced by the mutation, then consequently inflammation and replacement of muscle fibres by adipocytes or fibrotic tissue will not be as prevalent. This variability suggests that identification of the pathological features present in the muscle depends on the disease process and implies that certain components of the DAPC are more essential than others for the maintenance of muscle structure. However, it should also be noted that the nature of the gene inactivation in the mouse model affects the severity of the pathology – FKRP L276Ineo+/P448Lneo+, with its global knock-down in *Fkrp,* has replacement of muscle fibres with fibroadipogenic tissue, suggesting that it has a more severe pathology than $FKRP_{MD}$, which has *Fkrp* expression in the CNS and no obvious loss of muscle fibres.

In human patients, a number of muscular dystrophies have a characteristic pattern of muscle involvement, with calf hypertrophy associated with a number of neuromuscular conditions (Reimers *et al.* 1996) and further more specific patterns associated with certain conditions; for example, in the limb girdle muscular dystrophies, the most severely affected muscles are those of the upper and lower limb girdles. A specific pattern of muscle involvement has also been reported in the mdx mouse – although dystrophic pathology has been documented in various muscles throughout the animal, the extraocular (Porter *et al.* 1998), toe (Dowling *et al.* 2002) and intrinsic laryngeal muscles (Marques *et al.* 2007) seem to be relatively spared by the disease process, whereas contrastingly the diaphragm displays comparatively worsened histopathology. This suggests that the presence of dystrophin at the DAPC is not uniformly important for the integrity of each muscle in the body and these three muscles are most likely spared because they are not load-bearing, so consequently not subjected to marked contractile stresses. For most of the other models described in this review, a detailed description of the muscle involvement pattern was not provided in the publications, with the exception of the Large^{myd}, where all muscle groups were reported to be equally affected, with the exception of the tongue, which was spared, and the $FKRP_{MD}$ where the soleus was reported to be less affected than the gastrocnemius and diaphragm. So, in contrast to dystrophin deficiency, the Large^{myd} suggests that correctly glycosylated α-dystroglycan is uniformly important for all muscles. As this glycosylation arrangement on α-dystroglycan is crucial for binding to the basement membrane, it is most likely that this phenotype is associated with a loss of signalling at the DAPC, along with a reduction to contraction-induced injury. However, this is somewhat contrasted by the $FKRP_{MD}$ mouse reporting reduced pathology in the soleus, although this can most likely be attributed to the soleus, a postural muscle, being composed of mostly slow type I muscle fibres, which are more resistant to contraction-induced injury (Macpherson *et al.* 1996).

Interestingly, the phenomenon of worsened histopathology in the diaphragm is seen in a number of other mouse muscular dystrophy models – FKRP L276Ineo+/P448Lneo+, FKRP L276Ineo+/E310delneo+, FKRP P448Lneo+ and abdn$^{-/-}$ mice, but not the $FKRP_{MD}$ mouse. It is thought to occur because the diaphragm is the most used muscle in laboratory mice, therefore suffering from the most contraction-induced damage. Unfortunately, the pathology of the diaphragm compared to other hindlimb muscles has not been reported for all the models in this review. These data would be very interesting, as they would elucidate the importance of the different DAPC components for muscle resistance to contraction-induced injury.

By focussing this review on models with mutations/inactivations in the DAPC, we have selected models thought to be sensitive to contraction-induced damage to the muscle fibre membrane. There are a number of ways to assess this sarcolemmal damage in mice, such as evaluating the uptake of systemically administered Evans blue dye into muscle fibres (Hamer *et al.* 2002), with this phenomenon seen in the mdx (Matsuda *et al.* 1995), Sgca-null, gsg$^{-/-A}$, gsg$^{-/-B}$, adbn$^{-/-}$ and Scgb-null mouse models. Another method, which is also used in human patients, is assessment of MCK levels in the serum, with increased levels observed in human muscular dystrophy patients (Moat *et al.* 2013), the mdx mouse and the vast majority of the other muscular dystrophy models featured in this review, including Large^{myd}, Scgb-null, Large^{vls}, postnatal fukutin null, Myf5-Cre-FKTN-KO, Sgcd-null, MCK-Cre-FKTN-KO, Scga-null, FKRP L276Ineo+/P448Lneo+, gsg$^{-/-A}$, gsg $^{-/-B}$ FKRP P448L, dsg$^{-/-}$ FKRP P448Lneo+ mice, L276Ineo+/E310delneo+ and MCK-DG null. Given that all models, with the exception of the POMGnT1$^{-/-}$ mouse, displayed centrally nucleated fibres in histopathological analysis, indicating muscle regeneration following muscle fibre degeneration, it is not surprising that so many models display increased serum CK in the phenotype.

Measuring the levels of MCK in circulating blood is a crude, global measure of muscle fibre damage and does not provide any information about the specific pattern of muscle involvement or pathological progression of the disease. Instead, currently the most common method for evaluating the progression of muscle damage in human patients is by taking biopsies to evaluate the histology, which in itself is quite invasive and causes muscle damage. Therefore the research focus has been on finding new, alternative biomarkers for evaluating the progression of muscular dystrophy. Promising biomarkers are changes in the composition of certain muscles, detectable by magnetic resonance imaging (MRI) (Fan *et al.* 2014), and alterations in the levels of skeletal muscle-specific microRNAs (myoMirs) in the serum of mutants such as the mdx in comparison with control mice, which may mirror pathological processes occurring within

skeletal muscle (Roberts *et al.* 2012, 2013; Vignier *et al.* 2013). Further evaluation of disease progression in these other mouse models with inactivations/mutations in the other DAPC components will help to elucidate disease progression further, for example using miRNA biomarkers to identify whether and when crisis periods occur in other models and fMRI to explore the pattern of muscle involvement in these models.

Physical symptoms

A number of these mouse models are smaller than control littermates – Large^{myd}, Large^{enr}, FKRP$_{MD}$, POMGnT1 null, POMGnT1$^{-/-}$, fukutin chimera, dsg$^{-/-}$, Myf5-Cre-Fktn-KO, gsg$^{-/-}$ FKRP P448L-neo+, Large^{vls} and MORE-DG null. This could be due to reduced growth, a loss of body weight once the animal's phenotype worsens and it starts to lose muscle fibres and muscle mass, or a combination of the two. The IGF1-Akt signalling pathway is involved in regulation of skeletal muscle growth (reviewed in Schiaffino and Mammucari (2011)), and it is initiated by laminin binding to α-dystroglycan in the extracellular space, so a loss of this binding, seen in a number of these models, reduces Akt signalling and consequently muscle growth, contributing to smaller mutant mice (Langenbach & Rando 2002). Interestingly, these phenotypes are in marked contrast to the muscle-specific dystroglycan knockout and Sgca-null, which have increased weight compared to controls and the gsg$^{-/-B}$ mouse, which has no change in body weight compared to wild type. Similarly to mdx (Sharp *et al.* 2011), these models are reported to have a number of hypertrophic muscle fibres or an increased number of muscle fibres, so it is likely that the muscle fibre hypertrophy accounts for the increased body mass. Both the mdx and gsg$^{-/-B}$ models are reported to have increased levels of Akt activation (Peter & Crosbie 2006), demonstrating that loss of individual DAPC components differentially affects regulation of muscle size. Perhaps, a total loss of laminin binding to α-dystroglycan, as seen in the Large^{myd} or FKRP$_{MD}$, results in smaller mutant mice because there is little Akt activation, whereas a loss of other DAPC components not directly involved in binding (dystrophin or γ-sarcoglycan) perturbs regulatory or compensatory signalling pathways, leading to increased Akt activation.

However, in Scga-null mouse, the increased mass was reported as due to increased water and connective tissue content, rather than contractile material, suggesting that in reality, things are not as simple as postulated above. Given that dystrophin binds directly to the contractile apparatus, it is not surprising to assume that altered expression of dystrophin would change the arrangement or amount of contractile proteins in the muscle fibre. Contrastingly, the sarcoglycan complex has been shown to affect aquaporin-4 expression, suggesting that loss of sarcoglycan affects water regulation in muscle fibres (Assereto *et al.* 2008). Furthermore, the sarcoglycan complex is associated with dystroglycan (Brenman *et al.* 1995; Chan *et al.* 1998), providing a mechanism by which a loss of sarcoglycan expression might affect the composition or arrangement of the extracellular matrix. This suggests different roles for α-sarcoglycan and dystrophin with respect to the organization of the intracellular muscle environment.

Some muscular dystrophy mouse models also have physical abnormalities, with kyphosis reported in mdx (Laws & Hoey 2004), Large^{myd} and Large^{enr}. Additionally and unsurprisingly, motor function and coordination is affected, with a number of mutants, reported to lose their hindlimb extension reflex – Large^{myd}, POMGnT1-null, Large^{enr}, fukutin chimera, FKRP L276Ineo+/P448Lneo+, FKRP L276Ineo+/E310delneo+, FKRP P448L, FKRP P448Lneo+ and MORE-DG null. In some models, these abnormalities have been further characterized, with a motion tremor and delayed righting reflex identified in the MORE-DG null; reduced endurance in gsg$^{-/-}$; gait abnormalities in the Large^{myd}, gsg$^{-/-A}$ and fukutin chimera; reduced absolute force in Sgcb-null mice; a reduced specific force in mdx (Lynch *et al.* 2001), Sgca-null and Sgcb-null mice; reduced resistance to exercise in MCK-Cre-FKTN-KO, mdx (Dellorusso *et al.* 2001; Sharp *et al.* 2011) and Sgcd-null; and finally a reduction in forelimb grip strength identified in mdx (Connolly *et al.* 2001), Large^{enr}, Large^{vls}, postnatal fukutin null and Myf5-Cre-FKTN-KO. However, histological indicators of muscular dystrophy do not necessarily translate into functional impairments, with gsg$^{-/-}$ mice not having reduced resistance to eccentric exercise and abdn$^{-/-}$ mice not having a reduction in isometric force, specific force or reduced resistance to eccentric muscle contraction. This suggests that the different parts of the complex play different roles in the regulation of muscle function, with α-dystrobrevin less important than dystrophin or dystroglycan with respect to resistance of the muscle fibre to contraction-induced damage.

Finally, histopathological changes in the heart and/or functional evidence of cardiac dysfunction has been observed in the mdx (Bridges 1986) and some of the other mouse models – Scgd$^{-/-}$, Sgca-null, Sgcd-null, gsg$^{-/-B}$, Sgcb-null, Scgb-null, abdn$^{-/-}$ and gsg$^{-/-A}$ mice, but not others – FKRP L276Ineo+/P448Lneo+, FKRP L276Ineo+/E310delneo+ mice and FKRP P448Lneo+. This further supports the idea that despite the same complex being functionally compromised, there are different degrees of severity, affecting different tissues, depending on which part of the complex is inactivated.

Reproduction

Mdx mice, which are bred as homozygous females with hemizygous males, are exceptional breeders (http://jaxmice.jax.org/strain/001801.html), so the mutation appears to have no effect on the mouse's reproductive capability. The other muscular dystrophy models discussed here are generally recessively inherited, generated by breeding of two heterozygote mice, and so very few studies have reported the effect of these mutations on reproduction. Where it has been commented on Large^{myd}, Large^{enr}, POMGnT1 mutant and FKRP$_{MD}$, with the exception of the FKRP$_{MD}$, reproduction

has been affected by the mutation. Although in combination these data suggest that a loss of the signalling function at the DAPC through α-dystroglycan affects the reproduction phenotype, more studies on other mouse models are necessary to conclusively determine how, if at all, reproductive functioning is affected by loss or inactivation of the different DAPC components.

Lifespan

Although early work on mice up to 12 months of age suggested that mdx did not have a reduced lifespan (Carnwath & Shotton 1987), a more recent long-term study showed that mutants have a significantly shorter lifespan than C57Bl/10 mice (Chamberlain *et al.* 2007). Other models are also reported to have reduction in lifespan, including the MORE-DG null (very few animals survive beyond 4 weeks), Large^{myd} (mean lifespan 17 weeks of age, ranging from 5 to 37 weeks), gsg$^{-/-A}$, dsg$^{-/-}$ (50% of animals dying by 5 months of age), Myf5-Cre-FKTN-KO (78% of mutants die before 35 weeks of age) and Large^{enr} (average lifespan 6-8 months, with maximum of 11 months). Associated with this reduced lifespan, these models are also reported to have preweaning losses. Interestingly, a number of other models, including POMGnT1$^{-/-}$, POMGnT1-null, fukutin chimera and FKRP P448Lneo+, are reported to have a variable life expectancy, ranging from a few days to old age, suggesting a wide range of variation in phenotypic severity between individual animals. Contrastingly, there are other models that are not reported to have a reduced lifespan – FKRP$_{MD}$, gsg$^{-/-B}$, FKRP L276Ineo+/P448Lneo+, FKRP L276Ineo+/E310delneo+, abdn$^{-/-}$ and the MCK-DG null mouse. Some of these differences can be explained by the design of the models; for example, knocking out dystroglycan early in development (MORE-DG null) produces a markedly more severe phenotype than knocking it out just in the muscles later during development (MCK-DG null). Furthermore, as a number of the models with a reduced or variable lifespan have been shown to have cortical involvement, deaths in these animals, although connected to a loss of DAPC function, might be unrelated to dystrophic muscle pathology. However, it should be noted that in those where lifespan is not reported to be reduced, it might be that they simply have not looked at old enough mice or performed a survival study to demonstrate conclusively when the phenotype is lethal.

Disease modifiers

To study these different models further, a number of different transgenic crosses have been performed, leading to interesting and somewhat surprising results. It seems that the genetic background of the original mouse model plays an important role in modifying disease phenotype. Due to the nature of generating transgenic mouse models, a number of these mutants are on mixed genetic mouse backgrounds, but even when taking this into account, crossing mice onto the DBA2/J background worsens the disease phenotype, seen with both mdx (Fukada *et al.* 2010) and gsg$^{-/-}$ mice (Heydemann *et al.* 2005). This suggests that muscle development, structure and/or maintenance varies between the different laboratory mouse strains, which, whilst interesting, has implications for comparisons of different mouse models or studies, particularly those on mixed or different genetic backgrounds.

These mouse mutants are caused by reduced expression or inactivation of one of the components of the DAPC, so transgenic restoration of this component improves or ameliorates the phenotype, as shown with dystrophin in the mdx mouse (Cox *et al.* 1993a; Wang *et al.* 2000; Harper *et al.* 2002; Gregorevic *et al.* 2004) and large in the Large^{myd} mouse (Gumerson *et al.* 2013). However, as studies in the δ-sarcoglycan-deficient chimeric mice show (Vitale *et al.* 2012), a certain level of protein expression is required, with histological and functional improvement observed only when wild-type embryonic stem cell incorporation was over 60%. Interestingly, this is different to the mdx, where the phenotype was improved with <30% embryonic stem cell incorporation into mdx chimera (Stillwell *et al.* 2009), and furthermore, significant functional improvement in mdx is seen when only 20% of the fibres express dystrophin (Sharp *et al.* 2011). Furthermore, the level of protein expression needs to be controlled, because transgenic overexpression of γ-sarcoglycan induced a dystrophic phenotype in wild-type mice (Zhu *et al.* 2001). However, these mice were generated on a mixed background of BL6/DBA2 – as discussed earlier; the DBA2/J background is generally a poor background for muscle function, which would affect the results of this study. Again, these further highlight that the different components of the DAPC are not functionally equivalent and support the idea that the loss of individual proteins from the complex affects function in different ways.

Unsurprisingly, crossing two models with inactivations in two DAPC components together to create a double mutant worsens the phenotype, seen when the mdx was bred independently with the abdn$^{-/-}$ (Grady *et al.* 1999), Large^{myd} (Martins *et al.* 2013) and δ-sarcoglycan (Li *et al.* 2009) mice, or when the Scga-null mouse was bred with the Scgae-null mouse (Lancioni *et al.* 2011). Similarly, mdx mice that are also utrophin deficient exhibit a worsened pathology (Deconinck *et al.* 1997).

In muscular dystrophies where the function of the DAPC is perturbed, it is thought that a loss of adhesion between the muscle fibre and the extracellular matrix contributes to the phenotype, because the resulting muscle fibre is less resistant to contraction-induced membrane damage. However, the DAPC is not the only adhesion complex in muscle. A mutant with a double inactivation of the DAPC and integrin α7 (affecting the integrin adhesion complexes) had an exacerbated phenotype compared to the single gsg$^{-/-A}$ (Allikian *et al.* 2004) and mdx mutant mice (Rooney *et al.* 2006), whilst upregulation of integrin α7 (theoretically increasing muscle fibre adhesion) in the mdxutr$^{-/-}$ mouse increased the longevity of the mouse and reduced the dystro-

phic histopathology (Burkin *et al.* 2001). Furthermore, inactivation of dysferlin (a protein linked to skeletal muscle repair) through crossing the mdx mouse model with a dysferlin-null mouse created a double knockout with a worsened phenotype than either of the single-gene knockout mice (Han *et al.* 2011). However, contrastingly, transgenic upregulation of dysferlin (which would in theory increase the rate of membrane repair) (Millay *et al.* 2009) or integrin α7 (Milner & Kaufman 2007) did not improve the phenotype of Sgcd-null mice.

The precise glycosylation arrangement on α-dystroglycan is important for binding of DAPC to the basement membrane, anchoring the muscle fibre to the extracellular environment. Looking beyond the DAPC, the carbohydrate arrangement in muscle seems to be important for progression and development of the phenotype, with overexpression of Galgt2, a cytotoxic T cell GalNAc transferase, shown to improve the phenotype of both mdx (Martin *et al.* 2009) and Sgca-null mice (Xu *et al.* 2009). Of interest is the role of CMAH, a gene encoding CMP-Neu5Ac cytidine-5′-monophospho-N-acetylneuraminic acid hydroxylase, which generates Neu5GC, a common sialic acid modification in mammalian muscle (Varki 2010), with the exception of humans, where the function of this gene has been lost (Chou *et al.* 1998; Irie *et al.* 1998). The loss of this gene worsened the phenotype of both the mdx mouse model (Chandrasekharan *et al.* 2010) and the Scga-null mouse (Martin *et al.* 2013), perhaps contributing to the phenotype differences between mouse models of muscular dystrophy and the human phenotype.

Relevance to human conditions

Human conditions are caused by a variety of genetic changes that affect the synthesis or functioning of a protein – these can be whole-scale genetic changes, seen in DMD with deletions or duplications of dystrophin exons (Muntoni *et al.* 2003), or they can be point mutations in individual proteins, such as the L276I mutation in *Fkrp*, which is associated with LGMD2I (Brockington *et al.* 2001). Furthermore, recent studies have shown a complex relationship between genotype and patient phenotype demonstrating genetic heterogeneity along with a broad phenotypic spectrum associated with mutations in one single gene. For example, this can be seen in the dystroglycanopathies, where mutations in each gene have been associated with both severe congenital neuromuscular conditions and adult onset limb girdle muscular dystrophies (Godfrey *et al.* 2011).

Interestingly, in mice, introducing some of these genetic changes identified in human patients does not generate a disease phenotype, for example $FKRP^{Tyr307Asn+/+}$ (Ackroyd *et al.* 2009) and $\alpha\text{-sarcoglycan}^{His77Cys+/+}$ (Kobuke *et al.* 2008), with the phenotype emerging only once gene expression had also been reduced. Differences between mice and humans, particularly in their size and mechanisms of growth and regeneration [reviewed (Partridge 2013)], should thus be considered when using mice as models of human muscular dystrophies.

Conclusions

The functioning of the DAPC is essential for muscle integrity, and perturbation of this complex in mouse generates a dystrophic muscle phenotype. A vast number of different mouse models carrying mutations or inactivations in the different component of the DAPC show that interestingly, perturbing either different parts of the complex or the same part of the complex at different developmental time points generates a phenotype with slightly different characteristics, suggesting that some parts of the complex are more crucial than others for its functioning. However, it must be noted that all these models carry different mutations on different genetic backgrounds, with potential disease modifiers contributing to the phenotypic differences. Unsurprisingly, creating double-knockout models by either inactivating the function of more than one part of the DAPC or further affecting muscle function generates a mutant with a worsened phenotype, when compared to the original mice used in the cross. However, correcting or interfering with the disease process in another way, for example increasing membrane repair, does not improve disease phenotype, suggesting that perhaps the disease process is not as simple as first thought. Alternatively, with recent *in vitro* data identifying both novel proteins associated with dystroglycan and alterations in dystroglycan protein interactions in the absence of dystrophin (Yoon *et al.* 2012; Johnson *et al.* 2013), perhaps the structure of the DAPC and/or the arrangement of its binding partners varies far more widely than previously considered. Therefore, to fully explain the differences between the models highlighted here, we may need to rethink our view of DAPC structure and consequently function.

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