to maintain an independent life without any medication. Delegates were shown how dopamine release from transplants can be delineated using PET (Ref. 5). The recent identification of stem cells capable of generating a wide variety of cell phenotypes in the nervous system has also opened up new possibilities in this field (J. Frisen, Stockholm, Sweden). Stem cells can be found in the adult CNS, where a small population of the ependymal cells lining the ventricles divide slowly in an asymmetrical fashion to generate a group of cells that reside in the adjacent subventricular zone[6]. These can yield all the major classes of CNS cell phenotypes after further division, migration and differentiation. They can also be isolated, expanded in a culture dish in the form of free-floating cell clumps (neurospheres), and transplanted into the adult brain, where, in the same way as grafts of foetal nigral cells, they can integrate and differentiate into diverse cell types[7]. The signalling pathways required to direct differentiation into individual cell types have yet to be elucidated in detail, and will form an important area for research in the coming decade.

The future?

An informal poll of the participants suggested that this style of meeting is destined to thrive, with an excellent scientific programme (enhanced by efficient organization), the magnificent surroundings and the restorative effect of skiing between sessions. It is to be hoped that future winter schools also enjoy similar success.

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PERSPECTIVES ON DISEASE

The neurobiology of Duchenne muscular dystrophy: learning lessons from muscle?

Derek J. Blake and Stephan Kröger

Several forms of inherited muscular dystrophy are associated with brain abnormalities and cognitive impairment. One of the most common and severe of these diseases is Duchenne muscular dystrophy (DMD). Dystrophin, the product of the *DMD* gene, is found in neurones, where it is associated with the postsynaptic membrane. Cognitive impairment in individuals with DMD is thought to be due to an abnormality in the neuronal membrane that is caused by lack of dystrophin. Recent experimental evidence has provided valuable clues in our understanding of the complex molecular neurobiology of muscular dystrophy.

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DUCHENNE AND BECKER muscular dystrophies (DMD and BMD) are caused by mutations in the *DMD* gene, which is located on the short arm of the X chromosome. DMD is one of the most common genetic diseases in humans and affects approximately 1 in 3500 boys, whereas BMD is milder and affects 1 in 30 000 boys. Individuals with DMD show progressive muscle degeneration and usually die before the age of 30 from respiratory or cardiac-muscle failure. By contrast, individuals with BMD are less severely affected and can, in some cases, have a normal lifespan. Although the primary defect in DMD and BMD has been identified as a functional or quantitative deficit in dystrophin, a large intracellular protein, the precise function of this protein is not fully understood. In the brain, as well as in muscle, dystrophin is associated with a complex of proteins that span the membrane and effectively link the cytoskeleton to the extracellular matrix. Several of these proteins have been implicated in an increasing variety of cellular processes, including synaptogenesis and microbial pathogenesis. Furthermore, several novel dystrophin-associated proteins have been identified in the brain that are not components of the dystrophin complex in muscle. A number of different mechanisms have been proposed to account for the pathological changes in dystrophin-deficient muscle. Some of the most popular of these theories state that dystrophin is required to maintain the mechanical stability of muscle, that dystrophin clusters ion channels and that dystrophin is involved in Ca^{2+} homeostasis[1,2].

While muscle weakness is the primary cause of mortality in individuals with DMD, other organs are affected by the disease[3]. The idea that brain function is also abnormal in individuals with DMD was first realized in the latter half of the 19th century by the French physician, Duchenne de Boulogne. Duchenne described a seven-year-old boy with DMD as being dull and having

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poor language skills[4]. This observation is particularly remarkable because it encapsulates the major features of CNS involvement in DMD.

Brain function in DMD

It is well established that there is a non-progressive cognitive deficit in individuals with DMD and BMD (Ref. 3). Their full IQ scores are shifted approximately one standard deviation to the left of the population mean, such that 20–30% of individuals with DMD fall in to the mild-retardation category. Statistically, the range of IQ scores of individuals with DMD is normally distributed and has a single mode. This is particularly important because it excludes a single type of mutation, or the deletion of a second gene within one of the many large introns of the *DMD* gene, from being the cause of the cognitive deficit in these individuals. It is therefore generally accepted that the primary cause of cognitive impairment in DMD is the lack of functional dystrophin rather than a secondary consequence of the muscle disease. Furthermore, sib-pair analysis has shown that there is a direct correlation between the poor IQ scores of affected brothers in the same family, whereas unaffected sibs have normal intelligence[5]. The exact nature of the cognitive defect in DMD remains unresolved. However, several studies have highlighted deficits in verbal IQ, language and reading ability. At this point, it should be mentioned that psychometric analysis of individuals with diseases that affect locomotor activity is not entirely straightforward. Physical disability could explain poor performances in tasks that require motor coordination. Furthermore, the socio–economic constraints of having severely handicapped children could also bias test results. In order to take account of these potential biases, some of the most-informative studies have been conducted on cohorts of patients and controls with similar severities of musculo–skeletal handicap. In particular, researchers have compared the cognitive functions of individuals with DMD with those of patients with spinal muscular atrophy (SMA), another common and severe neuromuscular disorder[6–8]. In these studies, verbal IQ (VIQ) scores were significantly lower in the DMD group (VIQ = 81.6) compared with the SMA population (VIQ = 100.2). However, on non-verbal tests, the DMD and SMA populations performed equally well[8]. Detailed analysis of the reading abilities and processing skills of individuals with DMD highlighted disabilities that were similar to those of children with dysphonic dyslexia, a frequently occurring form of developmental dyslexia[9]. Therefore, because there is a strong verbal component to most IQ tests, the poor global IQ performance scores of individuals with DMD could be primarily attributable to deficits in VIQ.

There are only a few reports on the intellectual performance of individuals with BMD. In one recent study, North and colleagues described four boys with mental retardation and psychiatric disturbance, but no apparent muscle weakness[10]. These boys were subsequently diagnosed as having BMD. This study shows that cognitive disabilities can occur before the onset of muscle weakness and raises the intriguing possibility that some patients with X-chromosome-linked mental retardation might have mutations in the *DMD* gene.

Anatomically, the gross morphology of DMD-affected brains appears normal. However, structural changes in the brain have been detected in some individuals with DMD using sophisticated imaging techniques. Slight cerebral atrophy has been found in 20 of the 30 DMD cases examined by Yoshioka and colleagues[11]. The extent of this atrophy was correlated directly with age and low IQ. In a separate study, Jagadha and Becker found an increased incidence of neuronal loss and gliosis in DMD (Ref. 12). Interestingly, they also noted that three patients in their study had abnormal dendritic branching and arborization of cortical pyramidal neurones[12].

Major advances in the study of genetic disorders in humans are often made by generating animal models for the disease. A number of mouse strains with mutations in the *Dmd* gene, such as Dmd^{mdx} and Dmd^{mdx3Cv} mice (see Fig. 1a for details) are available and have facilitated the use of invasive techniques to study brain function and biochemistry[13]. Behavioural abnormalities such as retention impairment have been documented in mice that lack dystrophin[14]. However, Dmd^{mdx} and Dmd^{mdx3Cv} mice have normal spatial learning and hippocampal LTP (Refs 15,16). Thus, the absence of dystrophin in Dmd^{mdx} mice has little effect upon the hippocampal-dependent processes of learning and memory. While a direct comparison between the intellectual performances of individuals with DMD and behavioural abnormalities of Dmd^{mdx} mice is of limited scientific value, both have CNS abnormalities of the same aetiology, namely lack of dystrophin. Thus, mouse models of muscular dystrophy provide a useful model to study the role of dystrophin in the CNS.

The molecular genetics of DMD

The *DMD* gene was one of the first human genes to be cloned by positional cloning and is notable not only for its medical importance, but also for its size. To date, the *DMD* gene is the largest gene in the human genome and occupies approximately 2.5 Mb of the human X chromosome. This gene encodes dystrophin and a family of N-terminal truncated isoforms that are produced by activating independent promoters (Fig. 1a). 'Full length' dystrophin, or Dp427, is transcribed from three promoters. The M promoter is active in skeletal and cardiac muscle, the B promoter is active in the hippocampus and cortex, and the P promoter drives the synthesis of dystrophin in cerebellar Purkinje cells[17]. The different Dp427 isoforms are probably functionally equivalent because they differ only in the first few amino acids at the N terminus. The other dystrophin isoforms are transcribed from promoters within the *DMD* gene (Fig. 1).

Dystrophin is a member of the α-actinin–β-spectrin family of proteins. In common with these proteins, dystrophin has an N-terminal actin-binding domain and a large rod-domain that is composed of spectrin-like repeats (Fig. 1a). The C-terminal region of the protein contains several domains that define the dystrophin family of related proteins. This family is composed of dystrophin, utrophin, dystrophin-related protein 2 (DRP2), and α- and β-dystrobrevin.

Dystrophin is found at the plasma membrane in muscle and at the postsynaptic membrane of some neurones in the brain[18–20]. In muscle, dystrophin is associated with an oligomeric protein complex, the dystrophin-associated protein complex (DPC) that spans the plasma membrane (see Box 1 for details)[21,22]. This complex is thought to reinforce the muscle membrane, which protects it from the mechanical stress of contraction and relaxation[23]. Loss of dystrophin from the sarcolemma

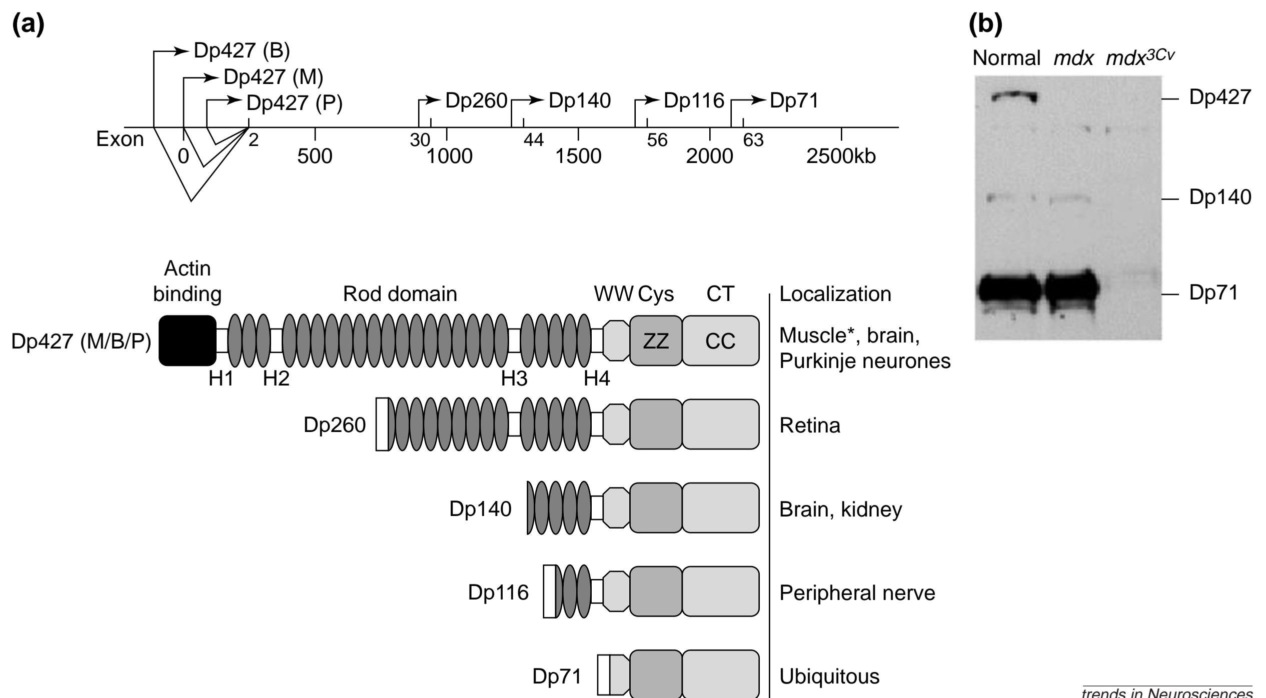


Fig. 1. ***Dystrophin and its isoforms.*** **(a)** *The location of the promoters within the dystrophin gene and the composition of the various dystrophin isoforms. Arrows indicate the positions of the seven promoters in the* DMD *gene. Three promoters drive the expression of 'full-length' dystrophin, Dp427. Each of these promoters [brain (B) muscle (M) and Purkinje neurones (P)] produce transcripts with a different 5′ untranslated region and first coding exon that are then spliced to exon 2. The other transcripts are produced from promoters located in the introns upstream of exons 30, 44, 56 and 63. The size of the* DMD *gene in kilobases (kb) is indicated. The transcriptional start site for the muscle promoter is considered to be the first base of the gene. Each dystrophin isoform can be represented modularly on the basis of its sequence composition, as depicted. The hinge regions (H1–H4) are proline-rich sequences that interrupt the rod domain. This domain is composed of 24 repeating units similar to those in β-spectrin that are predicted to form triple-helical coiled-coils. The C-terminal region contains a number of protein domains that form the binding sites for some of the components of the dystrophin-associated protein complex (DPC). These are the WW domain (WW), the ZZ domain (ZZ) that is located in the cysteine-rich region (Cys) and the coiled-coil domain (CC) in the C terminus (CT). The tissue distribution of the isoforms is indicated. The asterisk denotes that the Dp427-encoding transcript that is transcribed from the M promoter is found in skeletal and cardiac muscle.* **(b)** *A western blot of dystrophin and its isoforms in the brain. Dystrophin-immunoreactive proteins in mouse brains were detected by an antibody raised against the C-terminal region of dystrophin. In normal mice, dystrophin (Dp427), Dp140 and Dp71 are present. In the dystrophin-deficient* Dmd^{mdx} *mouse, there is a specific absence of dystrophin, whereas Dp140 and Dp71 are unaffected by the mutation. By contrast, in the brains of* Dmd^{mdx3Cv} *mice, which have severely reduced levels of dystrophin and all of the C-terminal isoforms, dystrophin and all the shorter isoforms are not detected.*

is coincident with a secondary reduction in the other components of the DPC (Ref. 24).

The DPC can be divided into three sub-complexes: the sarcoglycan complex, the dystroglycan complex and the cytoplasmic complex (Box 1). The genes for the sarcoglycans are expressed predominantly in skeletal, cardiac and smooth muscle[25]. Muscular dystrophy develops in mice that lack either α-, β- or γ-sarcoglycan[26–28]. In these mice, the levels of dystrophin and dystroglycan at the sarcolemma appear normal, suggesting that sarcoglycan deficiency contributes directly to the pathogenesis of muscular dystrophy. In humans, mutations in each of the sarcoglycan genes cause different forms of limb-girdle muscular dystrophy (LGMD). Interestingly, individuals with dystrophin-related LGMDs do not appear to be retarded. This observation is important because it supports the idea that primary dystrophin deficiency is the cause of the CNS abnormalities in DMD, and it is consistent with a role for the sarcoglycan complex that is ostensibly restricted to muscle.

The dystroglycan complex is synthesized ubiquitously and is involved in many cellular processes[29]. α- and β-dystroglycan are produced by proteolytic cleavage and glycosylation of a single precursor protein[30]. α-dystroglycan is a component of the extracellular matrix (ECM) that is required for the assembly of basal laminae and it is also a cell-surface receptor for a number of pathogenic micro-organisms[29,31]. (Dystroglycan-deficient mice die early in embryogenesis, owing to a disruption in the extra-embryonic basement membrane[32].) It also binds to several components of the ECM, including laminin 2 and laminin 4, agrin and perlecan. Interestingly, agrin and laminin 4 are required for neuromuscular junction (NMJ) formation. β-dystroglycan is a transmembrane glycoprotein that binds directly to dystrophin and α-dystroglycan (Box 1).

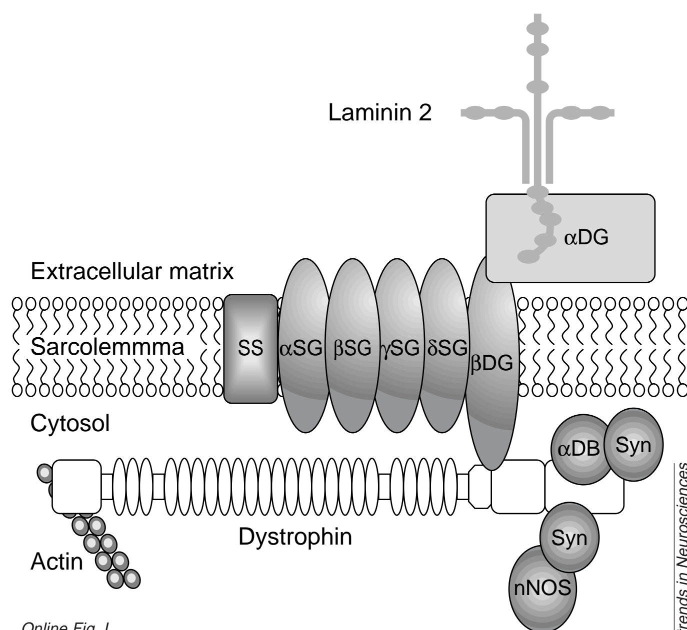
The cytoplasmic components of the DPC consist of the syntrophins (α-, β1- and β2-) and α-dystrobrevin. These proteins form a complex with the C terminus of dystrophin (Box 1). α-dystrobrevin-deficient mice develop muscular dystrophy without perturbing the assembly of the DPC at the sarcolemma, whereas mice that lack α-syntrophin are phenotypically normal[33,34]. These data, accompanied with a reduction in the level of cGMP in α-dystrobrevin-deficient muscle, suggest that α-dystrobrevin is involved in intracellular signalling[33]. Thus, the role of the DPC cannot be considered to be purely structural.

Three important points about the role of the DPC in skeletal muscle emerge. First, the individual components of the DPC contribute directly to the pathogenesis of muscular dystrophy. Second, the DPC is not merely a scaffolding complex but also has a role in cellular signalling. Third, some components of the DPC, such as the

Box 1. The dystrophin-associated protein complex in muscle

Dystrophin is associated with a protein complex, the dystrophin protein complex (DPC), which spans the muscle plasma membrane and effectively forms a molecular bridge between the muscle cytoskeleton and the extracellular matrix (reviewed in Ref. a). In muscle, the assembly of this complex is dependent on the presence of dystrophin. In dystrophin-deficient muscle, there is a secondary reduction in the levels of the DPC components at the sarcolemma. These alterations in the DPC are thought to contribute directly to the complex pathology in dystrophin-deficient muscle.

Several components of the DPC bind directly to dystrophin (Fig. I). Non-contractile F-actin binds to the N terminus of dystrophin and to additional sites within the rod domain (not shown)[b]. Most of the other dystrophin-associated proteins bind to the cysteine-rich and C-terminal domains of dystrophin. The interaction between β-dystroglycan (βDG) and dystrophin is dependent upon the WW domain and the EF-hand motifs in the cysteine-rich domain[c]. In addition to dystrophin, β-dystroglycan has also been shown to bind to the adapter protein, growth-factor-receptor-bound protein 2 (Grb2, not shown)[d]. This interaction might have a role in extracellular-mediated signal transduction, suggesting that the role of the DPC in muscle is not entirely structural. The final protein in the bridge between the cytoskeleton and the extracellular matrix is α-dystroglycan, which is associated with β-dystroglycan but is located in the extracellular matrix where it binds to some G-domain-containing proteins, including laminin 2, laminin 4, perlecan and agrin.



Online Fig. I

The sarcoglycan complex is composed of four glycoproteins, α- (50 kDa), β- (43 kDa), γ- (35 kDa) and δ- (35 kDa) sarcoglycan (SG), and a member of the tetrapsan family of proteins called sarcospan (25 kDa) (SS). Assembly and trafficking of the sarcoglycan complex to the membrane is dependent upon the co-synthesis of all four components[e]. This explains why the loss of one sarcoglycan component in limb-girdle muscular dystrophy (LGMD) leads to a secondary reduction in the levels of the other sarcoglycans. Although the sarcoglycans form a sub-complex it is not know whether any of them can bind directly to dystrophin.

The coiled-coil domain in the C terminus of dystrophin forms the binding site for the cytoplasmic components of the DPC (Ref. f). In muscle, there are three different syntrophin (Syn) proteins, α, β1 and β2, that can bind directly to dystrophin, α-dystrobrevin and utrophin. α-syntrophin also binds to neuronal NO synthase (nNOS) in muscle through reciprocal PDZ (PSD95, discs large, zona occludens) domains. Consistent with it being a dystrophin-associated protein, nNOS is lost from the sarcolemma of the dystrophin-deficient *Dmd*^{mdx} mouse and in patients with Duchenne and Becker muscular dystrophies[g]. However, nNOS appears to have no role in muscular dystrophy because the muscle pathology in nNOS-deficient *Dmd*^{mdx} mice is no worse than in *Dmd*^{mdx} mice[h]. Several other proteins that bind to the PDZ domain of syntrophin have also been identified (for clarity these have been omitted). These include, a family of voltage-gated Na^+ channels and stress-activated protein kinase 3 (Refs i,j).

As is the case with the syntrophins, there are multiple isoforms of α-dystrobrevin (αDB) in muscle[k]. However, in contrast to the syntrophins, each of the α-dystrobrevins is the product of the same alternatively spliced gene. The two largest isoforms, α-dystrobrevin 1 and α-dystrobrevin 2 are dystrophin-associated proteins[k,l]. The dystrobrevins also interact with syntrophin and dystrophin via their coiled-coil domains[m]. Thus, a quaternary complex of dystrophin, α-dystrobrevin and two syntrophin proteins is formed at the muscle membrane[n].

The postsynaptic membrane of the neuromuscular junction (NMJ) is a specialized sub-membranous site in muscle, which is characterized by a high concentration of ACh receptors and membrane invaginations. Many proteins that are involved in synaptogenesis are concentrated or found exclusively at the NMJ (Ref. o). Accordingly, the composition of the DPC at the NMJ differs from that in muscle. Dystrophin is replaced by the dystrophin-related protein utrophin. α-dystroglycan binds to agrin, a protein that coordinates synaptogenesis, and to the NMJ-specific laminin 4 isoform. Furthermore, β-dystroglycan has also been shown to bind to rapsyn, a protein that is required for the aggregation of ACh receptors, a key event in NMJ formation[p]. β2-syntrophin is restricted to the NMJ where it is thought to be associated with utrophin and the mictrotubule-associated serine/threonine kinase, MAST205 (Ref. q). The differences in the composition of the DPC at the NMJ suggest that the DPC has a role in synaptic transmission. Consistent with this idea, mice that lack utrophin have abnormal synaptic morphology, characterized by a reduction in junctional folding[o].

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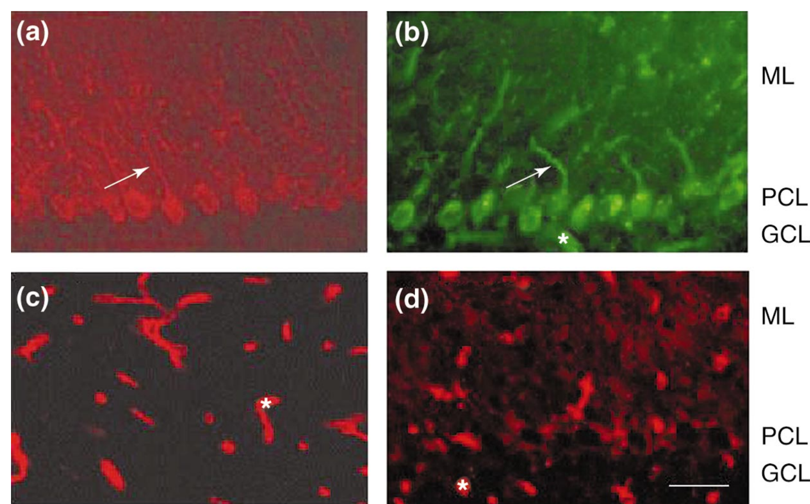

Fig. 2. ***Immunolocalization of dystrophin, Dp71 and the dystrophin-associated protein complex in the cerebellum****. Dystrophin* **(a)** *and α-dystroglycan* **(b)** *are present in cerebellar Purkinje cells. The Purkinje-cell soma in the Purkinje-cell layer (PCL), and dendrites (arrows) in the molecular layer (ML) are immunoreactive. There is punctate immunoreactivity of both proteins in the soma and dendrites whereas the nucleus is unstained. No dystrophin immunoreactivity is detected in the granule-cell layer (GCL), although α-dystroglycan is also found in perivascular astrocytes (asterisks) in all three layers. By contrast, Dp71 is present in perivascular astrocytes* **(c)**. *Other glial cells are not stained.* **(d)** *α-dystrobrevin 1 is also found in perivascular astrocytes (asterisks) but also stains some glial cells in the molecular layer. Note that the Purkinje-cell soma are unstained. Scale bar, 50 μm.*

sarcoglycans and α-dystrobrevin, might have specific, non-redundant roles in muscle. These findings are directly applicable to any proposal concerning the role of dystrophin in the CNS.

The dystrophin family in the brain

Dystrophin and several components of the DPC are found in the CNS. Dystrophin is found in the soma and dendrites of cortical and hippocampal neurones, and in cerebellar Purkinje cells (Fig. 2)[20]. Dystrophin is associated with the postsynaptic membrane of neurones and is present at high levels in postsynaptic densities (PSD), suggesting that it might have a role in synapse structure or function[20,35]. As shown in Fig. 1b, the dystrophin isoforms Dp71 and Dp140 are also found in the brain. These are thought to be associated predominantly with the microvascular glial cells (Fig. 2). Despite their abundance in brain, the C-terminal isoforms of dystrophin do not appear to be involved directly in the aetiology of mental impairment in individuals with DMD. As mentioned earlier, the distribution of IQ scores is unimodal and not correlated with any particular mutation. Furthermore, individuals with DMD who have mutations in the 5′ end of the *DMD* gene, have similar degrees of CNS involvement as do those with mutations in the 3′ end of the *DMD* gene[36]. However, as discussed later, the dystrophin isoforms Dp71 and Dp260 are involved in retinal neurotransmission.

Several components of the DPC, such as α-dystroglycan, α-dystrobrevin 1 and the laminin-α2 chain are associated with the cerebral microvasculature and are found at high levels in the glial–vascular interface (Fig. 2)[37]. The concentration of these proteins in perivascular astrocytes suggests that there could be a link between the ECM and the cytoskeleton of glial processes, similar to the link present in muscle. This interaction might be involved in stabilizing this interface, or it could form a diffusion barrier between the astrocyte and the endothelial cell. This is a plausible proposition because α-dystroglycan is involved in the adherence of some micro-organisms that infect the CNS (Ref. 29). α-dystroglycan is also located in neurones (Fig. 2), where it has a similar distribution to dystrophin (in the soma and dendrites of Purkinje cells[38]).

The cytoplasmic component of the DPC is widely distributed in the brain[39,40]. In addition to dystrobrevin and dystrophin, the syntrophins can bind to neuronal NO synthase (nNOS), a family of voltage-gated Na^+ channels and to a microtubule-associated serine/threonine kinase[41–43]. These interactions implicate the syntrophin–dystrobrevin complex in intracellular signal transduction. However, the presence of dystrophin in these complexes and the relevance of these interactions to the CNS phenotypes in DMD have yet to be established.

Recently, a novel member of the dystrobrevin family of proteins, β-dystrobrevin, was shown to be present in the brain[40]. β-dystrobrevin is related closely to α-dystrobrevin but is not found in muscle[40,44]. It is interesting for two reasons: (1) β-dystrobrevin binds directly to dystrophin and syntrophin, but is not a component of the DPC in muscle[40,44]; and (2) β-dystrobrevin, like dystrophin, is exclusive to neurones and is found at high levels in the PSD (Ref. 45). Thus, β-dystrobrevin might be a component of a neuronal DPC-like complex and, as is the case with many of the DPC components in muscle, could contribute to the molecular pathology of cognitive impairment in individuals with DMD. However, very little is known about the assembly of the DPC in DMD-affected brains.

A link with NO production?

As mentioned above, in brain and muscle, α-syntrophin can bind directly to nNOS (Refs 46,47). Furthermore, lack of either α-syntrophin or α-dystrobrevin in mice causes a marked reduction in the levels of nNOS at the sarcolemma[33,34]. The involvement of NO in activity-dependent neurotoxicity suggests that there could be a link between NO release and cognitive impairment in individuals with DMD. Central to the function of NO in neurones is the association of nNOS with the postsynaptic density proteins PSD95 and PSD93 (Ref. 41). PSD95 is involved in the anchoring and clustering of some NMDA-receptor subunits and Shaker-type K^+ channels[48]. The interaction between α-syntrophin and nNOS, and PSD95, nNOS and the NMDA receptor could implicate the dystrophin complex in some of the processes involved in excitatory neurotransmission. Furthermore, the interaction between the NMDA receptor and PSD95 is necessary for the coupling of Ca^{2+} release to NO production[49]. When expression of the gene for PSD95 is supressed using antisense in cultured neurones, the excitotoxicity triggered by Ca^{2+} release through the NMDA receptor is attenuated. The proximity of this complex to the NMDA receptors, and whether nNOS is bound to syntrophin and dystrophin at the postsynaptic membrane are currently unknown. However, it remains an intriguing possibility that abnormalities in NO production could contribute to the neuropathology of DMD.

Several abnormalities have been identified in dystrophin-deficient brains that could be related to alterations in pathways that involve NO. The enhanced susceptibility of dystrophin-deficient neurones to hypoxia-induced loss of synaptic transmission suggests that Ca^{2+}-dependent pathways of neurotoxicity might

have a role in the CNS pathology seen in DMD (Ref. 50). Furthermore, cultured cerebellar granule-cell neurones from *mdx* mice (these mice lack only Dp427) have 24% more free Ca^{2+} compared with those from normal mice[51]. These results could indicate that similar abnormalities in Ca^{2+} homeostasis in dystrophin-deficient muscle are also reflected in neurones. The association of syntrophin with nNOS, coupled with raised intracellular Ca^{2+} levels, might even render dystrophin-deficient neurones particularly sensitive to metabolic insults. These biochemical alterations could ultimately lead to impaired synaptic transmission or even neuronal death (see above).

Retinal involvement in DMD

One of the best-characterized CNS phenotypes of DMD has been described in the visual system. Although clinically the visual functions of individuals with DMD appear entirely normal, they show an altered response to a brief flash of light under dark-adapted (scotopic) conditions, as measured by the electroretinogram (ERG)[52–54]. The amplitude of the b-wave is reduced and its implicit time is delayed significantly. As the ERG of mice that lack Dp427 appears to be normal, absence of the smaller dystrophin isoforms is probably responsible for the altered ERG. Mice carrying a targeted deletion of exon 52 of the *Dmd* gene, which leads to a loss of Dp427, Dp260 and Dp140 (Fig. 1a), have a prolonged implicit time of the b-wave, but no change in its amplitude[55]. This indicates that some isoforms of dystrophin are responsible for different components of the visual response.

A reduction of the amplitude of the ERG b-wave under scotopic conditions is caused by impaired synaptic transmission between rod photoreceptors and their postsynaptic target cells in the retinal outer plexiform layer (OPL)[56]. Accordingly, dystrophin and dystroglycan immunoreactivity is concentrated in the retinal OPL of humans, mice and other species (Fig. 3a,b)[53,57,58]. Electron microscopy has revealed dystrophin in finger-like extensions in the distal-most part of the photoreceptor terminals, suggesting that dystrophin influences retinal synaptic transmission on the presynaptic side of the ribbon synapses[59–61]. The intracellular mechanism by which the absence of dystrophin causes impaired synaptic transmission in the photoreceptor terminals remains to be determined. As dystrophin and dystroglycan are not directly associated with the active zone of the ribbon synapses, it is most likely that mutations in dystrophin affect synaptic transmission indirectly.

Dystrophin and dystroglycan are present in the terminals of retinal rods and cones (Fig. 3a,b). This result is surprising, as the ERG deficit is mainly found under dark-adapted testing conditions, predicting an effect primarily on the rod photoreceptor pathway. One possible explanation for this discrepancy was found when the DPC was analysed in the retinae of *Dmd*^{mdx3Cv} mice. These mice have a splicing mutation in intron 65 of the *Dmd* gene that leads to a severe reduction in the levels of all the dystrophin isoforms in skeletal muscle, brain and retina. *Dmd*^{mdx3Cv} mice have an altered ERG that is similar to that seen in individuals with DMD. In the retinae of these mice, levels of dystrophin and dystroglycan are reduced severely, and have an altered distribution in rod terminals, whereas cone photoreceptor terminals are affected only mildly[60]. While this selective effect on rods agrees well with the observed alterations in the ERG, the mechanism by which mutations in dystrophin cause the impaired synaptic transmission in the retinal OPL remains to be determined. Interestingly, the *Dmd*^{mdx3Cv} mutation does not affect the total number of photoreceptors nor the overall morphology of the retina or of several retinal cell types, including photoreceptors. Therefore, in contrast to muscle, lack of dystrophin and its isoforms does not cause degenerative events in the retina and is, thus, not likely to be responsible for the mechanical stability of retinal cells. It seems more likely that the influence of dystrophin and the DPC on retinal information processing is more subtle. Another question that remains unanswered is why the distribution and function of dystrophin in cones is much less affected compared with dystrophin in rods, although both types of photoreceptors possess dystrophin and the DPC in their terminals? One possibility is that compensatory mechanisms are more prevalent in cones than in rods. Elucidating the function of dystrophin and the DPC in the retina might lead to a better understanding of the function of these proteins in the CNS and in particular at synapses.

Other muscular dystrophies with CNS involvement

In this article, the neurobiology of DMD and BMD has been discussed primarily. However, several other forms of muscular dystrophy are associated with brain abnormalities and cognitive impairment. Merosin-negative congenital muscular dystrophy (CMD) is caused by mutations in the laminin $\alpha2$ chain and is frequently associated with CNS abnormalities[62]. Laminin $\alpha2$ is the alpha chain of laminin 2, an ECM protein that binds with high affinity to α-dystroglycan (Box 1). Thus, two forms of muscular dystrophy that are associated with brain dysfunction are caused by defects in the DPC. These findings raise the interesting possibility that other forms of myopathy-associated brain abnormalities might be caused by defects in proteins associated with the DPC or ECM. Individuals with Fukuyama congenital muscular dystrophy (FCMD) are profoundly retarded and have brain abnormalities. This muscular dystrophy is caused by a lack of fukutin, a secreted protein with no ascribed function[63]. Individuals with FCMD have defects in neuronal migration that could reflect abnormalities in the basal laminae[64]. There are also several other forms of muscular dystrophy associated with mental retardation for which the genes have not been identified. These include Walker–Warburg syndrome and muscle–eye–brain disease[62]. Notably, the cloning of the genes that encode the different DPC components from muscle led rapidly to the identification of sarcoglycan mutations in individuals with LGMD. This raises the exciting possibility that proteins associated with dystrophin in the brain might be involved in the complex neurobiology of cognitive dysfunction.

Future perspectives and concluding remarks

Although little is known about the molecular mechanisms that cause cognitive impairment in individuals with DMD, considerable progress has been made in identifying the proteins and biochemical pathways that might contribute to this abnormality. Although dystrophin was identified more than a decade ago, the exact function of this protein in muscle remains unresolved. The lessons learnt from muscle are clearly applicable to the role of dystrophin in the CNS. It is

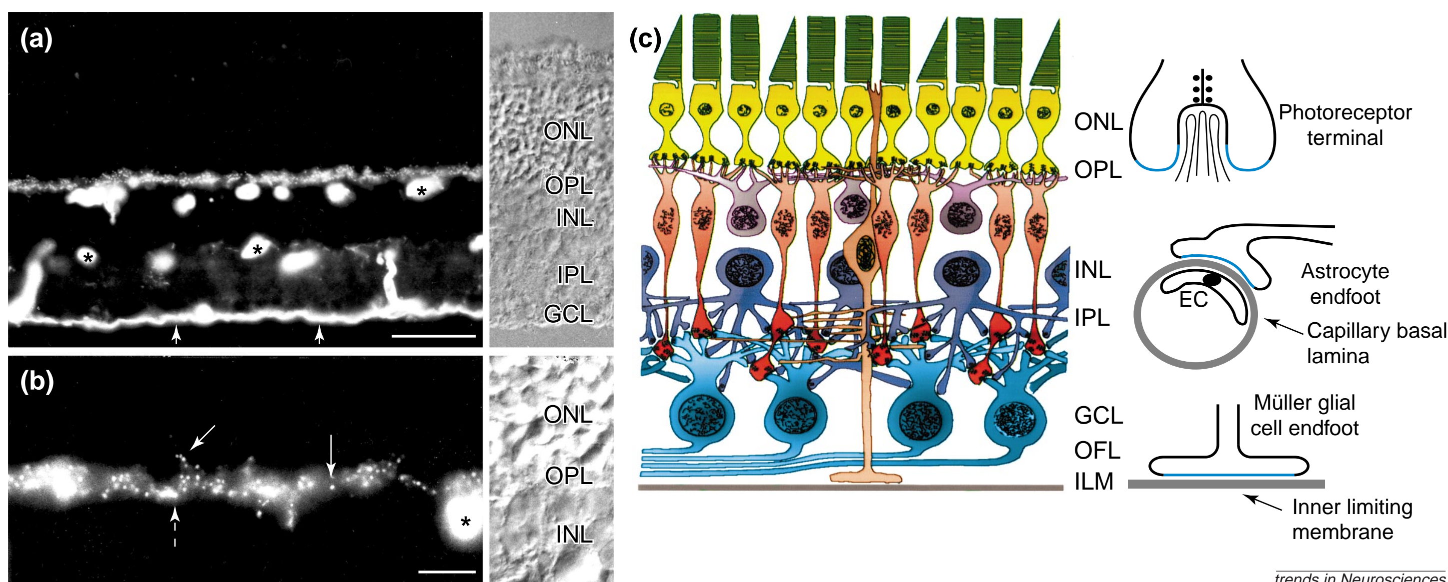

Fig. 3. ***Distribution of dystrophin and the dystrophin-associated protein complex (DPC) in the vertebrate retina.*** *Immunohistochemical analysis detects the DPC (as revealed by anti-α-dystroglycan immunoreactivity) at three locations in the mouse retina. The DPC is concentrated in the outer plexiform layer (OPL), around blood vessels [asterisks in* **(a)** *and* **(b)***], and at the vitreal border of the retina [arrows in* (a)*]. Analysis of the OPL at high magnification reveals that staining is concentrated in punctata with a diameter of approximately 0.5 μm [unbroken arrows in* (b)*] and in bands with a width of approximately 3 μm [broken arrow in* (b)*], which correspond to the terminals of rods and cones, respectively. Corresponding parts of the figures in Nomarski optics are shown on the right to indicate the various retinal layers.* **(c)** *shows a schematic representation of the laminar structure of the retina. The various retinal cell types are coloured as follows: photoreceptors, yellow; horizontal cells, purple; bipolar cells, red; amacrine cells, dark blue; ganglion cells, light blue; Müller glial cell, brown. The subcellular distribution of the DPC in the retina, as determined by immunoelectron microscopy, is indicated on the right side in blue. The immunoreactivity detected in the OPL reflects a concentration of dystrophin and dystroglycan in the extensions of rod and cone photoreceptor terminals. The DPC is not associated with the dendrites of the postsynaptic bipolar and horizontal cells, or with the active zone of the photoreceptor ribbon synapses. Blood-vessel-associated immunoreactivity is due to a concentration of dystroglycan in the endfeet of perivascular astrocytes in the region of contact to the capillary basal lamina. Immunoreactivity at the border between the neural retina and the vitreous reflects a concentration of the DPC in the endfeet of Müller glial cells at the contact site to the inner limiting membrane (ILM), a basal lamina that separates the neural retina from the vitreous humour. Dystroglycan is co-localized with dystrophin in the OPL, and with utrophin and Dp71 in the endfeet of astrocytes and of Müller glial cells. Abbreviations: EC, endothelial cell; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; OFL, optic fibre layer; ONL, outer nuclear layer. Scale bars, 25 μm in* (a) *and 10 μm in* (b).

now becoming accepted that the DPC might have a role in intracellular signalling. These findings could suggest non-mechanical roles for dystrophin in neurones. Analysis of the DPC in the retina and brain has also suggested that dystrophin could function at both the presynaptic and postsynaptic membrane. Thus, the absence of dystrophin in the CNS might primarily affect synaptic transmission. It is hoped that analysing dystrophin and the DPC in the CNS might not only help us to understand information processing in the brain but might also lead to better ways of treating the disease in muscle.

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