Zebrafish models flex their muscles to shed light on muscular dystrophies

Joachim Berger[1],* and Peter D. Currie[1],*

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Muscular dystrophies are a group of genetic disorders that specifically affect skeletal muscle and are characterized by progressive muscle degeneration and weakening. To develop therapies and treatments for these diseases, a better understanding of the molecular basis of muscular dystrophies is required. Thus, identification of causative genes mutated in specific disorders and the study of relevant animal models are imperative. Zebrafish genetic models of human muscle disorders often closely resemble disease pathogenesis, and the optical clarity of zebrafish embryos and larvae enables visualization of dynamic molecular processes in vivo. As an adjunct tool, morpholino studies provide insight into the molecular function of genes and allow rapid assessment of candidate genes for human muscular dystrophies. This unique set of attributes makes the zebrafish model system particularly valuable for the study of muscle diseases. This review discusses how recent research using zebrafish has shed light on the pathological basis of muscular dystrophies, with particular focus on the muscle cell membrane and the linkage between the myofibre cytoskeleton and the extracellular matrix.

Introduction

Muscular dystrophies (MDs) are a heterogeneous group of genetic diseases that affect different muscle groups, depending on the specific form of dystrophy. The genetic causes of more than 30 genetically distinct types of MD have been identified thus far (Bushby et al., 2009; Kaplan, 2011). The progressive degeneration of dystrophic muscles in these disorders is characterized by: abundant small-diameter fibres with central nuclei (indicating regeneration), infiltration of mononucleate cells, accumulating fibrosis and a broader variation in the size of myofibre cross-sectional areas compared with muscle in healthy individuals. In severe MDs such as Duchenne MD (DMD), affected individuals demonstrate muscle weakness from early childhood onwards, become wheelchair-dependent in their early teens and usually die in their early thirties from lung or heart failure. DMD is caused by mutations in dystrophin that lead to defects in the dystrophin-associated glycoprotein complex (DGC), which forms a link between the cytoskeleton of the muscle fibre and the extracellular matrix (ECM) (Fig. 1). Deficiencies in other components of the DGC, such as laminin-2, can elicit MD with similarly devastating symptoms (Fig. 1). On the other side of the clinical spectrum are MDs with very mild symptoms, as shown by individuals suffering from Becker MD (BMD), who in rare cases can be ambulant even late in life (England et al., 1990). Interestingly, BMD is caused by mutations that lead to only partial loss of dystrophin function. Other MDs are caused by deficiencies in the second major sarcolemmal complex, which mediates adhesion through integrins (Fig. 1). Sarcolemma-spanning integrins bind to laminin on the outside of myofibres and interact with actin on the cytosolic side, thereby forming a link between the myofibre cytoskeleton and the ECM.

In addition to pharmacological approaches, different therapeutic avenues for MDs are being explored, including stem cell transplantation, gene therapy based on delivery of full-length or truncated dystrophin, and remodelling of mutated transcripts by antisense oligonucleotides (Pichavant et al., 2011). Despite remarkable advances, however, no curative treatment for any form of MD exists. To discover the genetic basis of as-yet-unidentified MDs and to further develop treatments, the establishment of animal models that can be studied more efficiently and to a greater extent than affected humans is vital.

Over the past two decades, the zebrafish (*Danio rerio*) has received tremendous attention owing to its advantages as a model system. The rapid external development of easily manipulatable translucent embryos together with the efficient husbandry has sparked the establishment of many models of human diseases in zebrafish, and these models often closely resemble the human condition (Lieschke and Currie, 2007). The unique combination of these advantages makes the zebrafish particularly suited for the study of muscle. Importantly, as demonstrated by synteny analysis and cross-species mutant rescue experiments (Catchen et al., 2011), the transcriptional network that activates teleost myogenesis is conserved, and orthologues of the vast majority of human MD disease genes can be identified in the zebrafish genome (Steffen et al., 2007).

In addition to zebrafish models of MDs (listed in Table 1), which facilitated many exciting insights into MD pathologies, studies of chaperones and heat-shock proteins that assist the assembly of the contractile apparatus (Etard et al., 2007; Wohlgemuth et al., 2007; Hawkins et al., 2008; Sarparanta et al., 2012) have also shed light on the basic biology of muscle cell formation and maintenance. However, this review focuses on the recent contributions of zebrafish models to our understanding of MDs that

[1]Australian Regenerative Medicine Institute, EMBL Australia, Monash University, Clayton, Victoria 3800, Australia
*Authors for correspondence (joachim.berger@monash.edu; peter.currie@monash.edu)
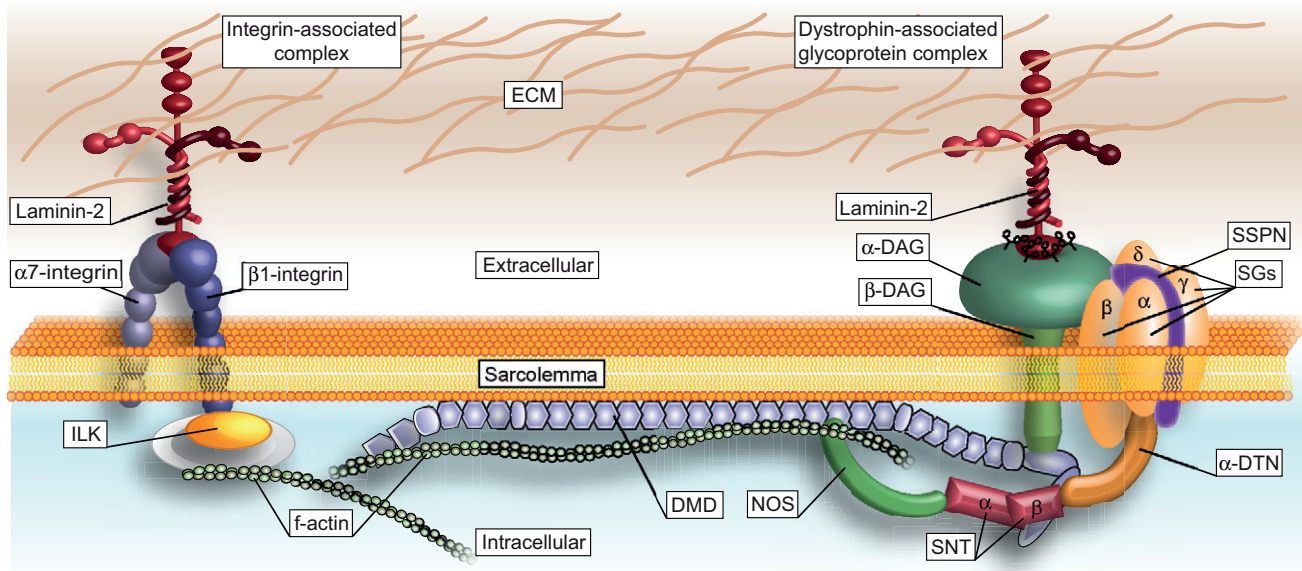
Fig. 1. Two protein complexes constitute the main mechanical link between the actin cytoskeleton and the ECM. Owing to their pivotal role in muscle integrity, deficiencies in components of the integrin-associated complex (left) or the dystrophin-associated glycoprotein complex (DGC; right) can result in MDs. DAG, dystroglycan; DMD, dystrophin; DTN, dystrobrevin; ILK, integrin-linked kinase; NOS, nitric oxide synthase; SG, sarcoglycan; SNT, syntrophin; SSPN, sarcospan.

result from defects in the sarcolemma and the ECM.

Modelling DMD and BMD

In general, a complete loss of dystrophin function results in DMD (OMIM 310200), one of the most severe and common forms of MD, and partial loss leads to the milder BMD (OMIM 300376). Nearly three decades of research on the dystrophin-deficient mouse have provided valuable insights into the biochemical function of dystrophin. Dystrophin is one of the core components of the DGC (Fig. 1) and plays a pivotal role not only in transferring the contraction force from the contractile apparatus to the ECM, but also in cell signalling, membrane stabilization and Ca^{2+} influx (Markert et al., 2011). Despite the detailed knowledge available on dystrophin function, no resolutive cure has been developed, partially due to the fact that dystrophin-deficient mice fail to recapitulate the clinical severity of DMD in humans (Hoffman et al., 1987; Kang, 2011). Current management of DMD is mainly based on administration of corticosteroids, which slow disease progression but do not constitute a cure.

Zebrafish models of DMD

Dystrophin deficiency has been modelled both by morpholino knockdown and by using identified null mutations in zebrafish

dystrophin (Bassett et al., 2003; Guyon et al., 2003). The zebrafish dmd^{ta222a} mutant (synonyms: *sapje*, *sap*) was isolated in a forward genetic screen via its reduction in birefringence, a quantifiable effect easily detectable under polarized light (Fig. 2B,B′) (Granato et al., 1996; Berger et al., 2012). This dystrophic mutant was subsequently identified as a carrier of a dystrophin null allele (Bassett et al., 2003); other zebrafish mutant alleles were later identified (Guyon et al., 2009; Berger et al., 2011). Subsequent comparison of the dystrophin-deficient zebrafish to humans with DMD revealed that it closely resembles the human condition in severity and progression (Berger et al., 2010). In zebrafish, as in humans, dystrophin is initially expressed at the peripheral ends of the myofibres and gradually shifts to non-junctional sites (Wessels et al., 1991; Clerk et al., 1992; Chambers et al., 2001; Berger et al., 2010). In both species, dystrophin loss progressively leads to abundant necrotic myofibres being replaced by mononucleate infiltrates, extensive fibrosis accompanied by inflammation, and broader variation in the size of muscle fibre cross-sectional areas (compared with healthy muscle) (Fig. 2C,C′). Furthermore, the lack of muscle progenitor proliferation to compensate for the extensive skeletal muscle loss, as has been suggested to occur in humans, is paralleled in zebrafish (Berger et al., 2010). An interesting finding

from the study of *dmd* zebrafish is that dystrophin-deficient mutants that are anaesthetised with Tricaine, an anaesthetic that blocks sodium channels, do not display detached myofibres. On removal of the anaesthetic, however, fibre detachment can be observed in real time (Berger et al., 2010). Although the precise mechanism that leads to muscle injury requires further investigation, generation of muscle force seems to play a crucial role in triggering myofibre detachment, at least in zebrafish. Such in vivo observations obtained with zebrafish demonstrate that using the advantages of different model systems leads to novel insights into the onset and progression of disease pathologies.

Dystrophin-deficient zebrafish contribute to the exploration of potential therapies for DMD

Currently, the most promising therapeutic approach for DMD is the administration of antisense oligonucleotides that alter the splicing of dystrophin pre-mRNA transcript, thereby excluding targeted exons from the mature mRNA and partially restoring functional protein (Muntoni and Wood, 2011). Although many uncertainties arising from ongoing clinical studies remain (van Deutekom et al., 2007; Kinali et al., 2009), statistically evaluated analysis of exon skipping in dystrophin-deficient zebrafish

Table 1. Zebrafish models of monogenic neuromuscular diseases

Zebrafish gene(s)	Modelled human disease	Model type	Reference
cav3	Multiple types	Morphants	Nixon et al., 2005
col6a1, col6a3	Ullrich syndrome, Bethlem myopathy	Morphants	Telfer et al., 2010
dag1	Recessive limb-girdle MD with primary α-dystroglycan defect	Morphants	Parsons et al., 2002
		Missense mutant	Gupta et al., 2011
desma	Myofibrillar myopathy, dilated CM 1I	Morphants	Vogel et al., 2009
dmd	Duchenne MD	Morphants	Guyon et al., 2003
		Null mutants	Bassett et al., 2003; Berger et al., 2011
		Splice mutant	Guyon et al., 2009
dnajb6b	Limb-girdle MD 1D	Morphants	Sarparanta et al., 2012
dysf	Multiple types	Morphants	Kawahara et al., 2011a; Roostalu and Strahle, 2012
fkrp	Multiple types	Morphants	Thornhill et al., 2008; Kawahara et al., 2010; Lin et al., 2011; Wood et al., 2011
fktn	Multiple types	Morphants	Lin et al., 2011; Wood et al., 2011
ilk	Dilated CM due to ILK defect	Null mutant	Knoll et al., 2007; Vogel et al., 2009
ispd	Walker-Warburg syndrome	Morphants	Roscioli et al., 2012
itgα7	Congenital MD with integrin defect	Morphants	Postel et al., 2008
lama2	Congenital MD with merosin deficiency	Null mutant	Hall et al., 2007
lama4	Dilated CM due to laminin α4 defect	Morphants	Pollard et al., 2006; Knoll et al., 2007
lamb2	Congenital myasmatic syndrome with laminin β2 deficiency	Missense mutant	Jacoby et al., 2009
lmna	Multiple types	Morphants	Vogel et al., 2009
pomt1, pomt2	Multiple types	Morphants	Avsar-Ban et al., 2010
ryr1b	Multiple types	Splice mutant	Hirata et al., 2007
sepn1	Multiple types	Morphants	Deniziak et al., 2007; Jurynec et al., 2008
sgcd	Limb-girdle MD 2F, dilated CM	Morphants	Guyon et al., 2005; Cheng et al., 2006; Vogel et al., 2009
tcap	Limb-girdle MD 2G, congenital MD with telethonin defect	Morphants	Vogel et al., 2009; Zhang et al., 2009
tnnt2a, tnnt2c	Familial hypertrophic CM 2, dilated CM 1D	Mutants, morphants	Sehnert et al., 2002; Huang et al., 2009; Vogel et al., 2009
		Morphants	Ferrante et al., 2011
tnni2a.4	Sheldon-Hall syndrome	Null mutant	Ferrante et al., 2011
tnnt3a, tnnt3b	Sheldon-Hall syndrome	Morphants	Ferrante et al., 2011
ttn	Multiple types	Linked mutant	Steffen et al., 2007a
vcl	Hypertrophic CM with vinculin deficiency, dilated CM 1W	Morphants	Vogel et al., 2009

CM, cardiomyopathy.

demonstrated that 20-30% of normal dystrophin transcript levels are needed to recover a severe dystrophic pathology (Berger et al., 2011). Issues regarding systemic antisense oligonucleotide administration in individuals with DMD, however, have not been addressed by this study, because oligonucleotides were injected into the yolk sack of 0.5-hour-old zebrafish embryos.

The advantages of the zebrafish were also employed in a small-molecule screen that identified 19 compounds that prevented dystrophic pathology in dystrophin-deficient zebrafish (Kawahara et al., 2011b). Zebrafish can also be used to analyze lead drugs such as PTC124, which was claimed to promote read-through of premature stop codons in the dystrophin gene (Welch et al., 2007), a claim that was subsequently disputed (Auld et al., 2010). In addition, our own data show consistently that administration of PTC124 up to toxic levels has no effect on the phenotype of dystrophin-deficient zebrafish (our unpublished results). Again, these findings demonstrate the value of the zebrafish model for fast and statistically evaluated testing of potential therapies.

Modelling dystroglycanopathies
The dystroglycan (*DAG1*) gene encodes α- and β-dystroglycan, which are core components of the DGC (Fig. 1). In this complex, α-dystroglycan needs to be glycosylated to bind to laminin-2, in this way linking the subsarcolemmal cytoskeleton to the ECM. Several patients have been reported that have aberrant glycosylation of α-dystroglycan that leads to a reduced binding of α-dystroglycan to laminin-2 (Godfrey et al., 2011). These individuals display a wide range of muscle symptoms, and their conditions are commonly referred to as dystroglycanopathies.

Mutations in *dag1*
To date, only one patient with a missense mutation in *DAG1* has been reported (Hara
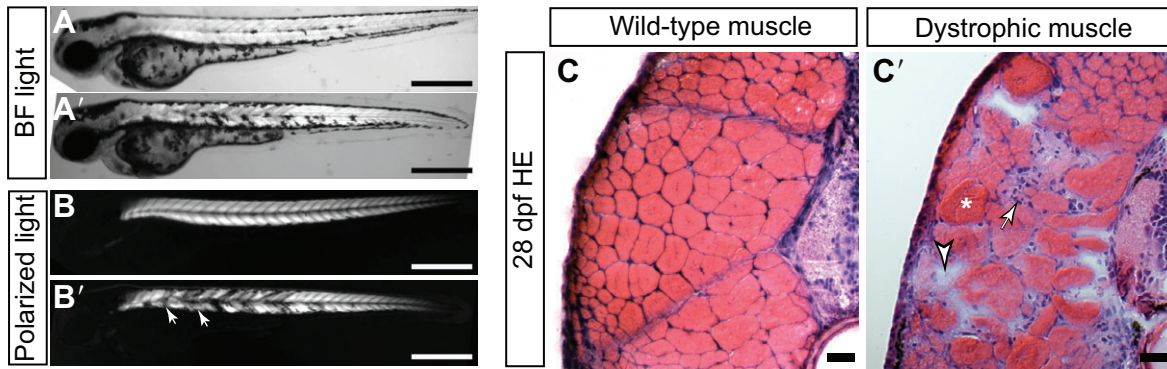
Fig. 2. Characteristics of dystrophic muscle in zebrafish. At 3 days post fertilisation (dpf), it is challenging to distinguish a wild-type larva (A) from a dystrophic larva (A′) under bright-field conditions (apart from the marked misplaced pigment cells of the dystrophic mutant). In contrast, birefringence effectively visualizes the myofibril under polarized light, whereby somites with detached myofibres of dystrophic mutants (B′) appear darker (indicated by arrows) than wild-type somites (B). Cross-sections of 28-dpf-old wild-type (C) and dystrophin-deficient (C′) larvae stained with H&E (HE). As in humans, the dystrophin-deficient muscle of the *dmd* mutant is characterized by myofibre atrophy (arrowhead), infiltration of mononucleate cells (arrow), eosinophilic myofibres (asterisk) and broader variation of myofibre cross-sectional areas. Scale bars: 0.5 mm.

et al., 2011). *Dag1* null mice are early embryonic lethal owing to disruption of an extraembryonic basement membrane, the rodent-specific Reichert's membrane, which controls materno-embryonic exchange upon in utero fetal implantation (Williamson et al., 1997). In contrast to the mouse model, the external development of zebrafish embryos circumvents the early embryonic lethality seen in the rodent, thereby allowing the study of the role of the orthologous *dag1* gene in muscle integrity. In a morpholino-based knockdown study of the zebrafish *dag1* gene, Parsons and colleagues demonstrated the pivotal role of dystroglycan for muscle integrity (Parsons et al., 2002). More recently, two zebrafish loss-of-function *dag1* mutants have been described: the *dag1^{hu3072}* mutant contains a premature stop codon, and the *patchytail* mutant contains a missense mutation; both lack α- and β-dystroglycan protein (Gupta et al., 2011; Lin et al., 2011). In contrast to *dag1^{hu3072}* mutants, in which myofibres detach from myosepta and subsequent sarcolemmal rupture has been described (Lin et al., 2011), *patchytail* mutants only display widened myosepta (Gupta et al., 2011); these differences reflect the broad variation of clinical aspects seen in human dystroglycanopathies.

Interestingly, morpholino-based knockdown of maternal and zygotic contributions of dystroglycan leads to complete loss of dystrophin protein (Lin et al., 2011). Dystrophin is also absent from many fibres in dystroglycan-deficient chimaeric mice (Côté et al., 1999), and

deletion of the β-dystroglycan-binding region in dystrophin leads to loss of dystroglycan (Fritz et al., 1995; Rafael et al., 1996), reinforcing the notion that dystrophin and the dystroglycans are necessary for the assembly and stability of the DGC.

Secondary dystroglycanopathies are evoked by altered glycosylation of dystroglycan

As mentioned above, defective glycosylation of α-dystroglycan can abolish binding to laminin-2, thereby resulting in MD. Over the past few years, genes involved in the glycosylation of α-dystroglycan have been reported, namely *POMT1*, *POMT2*, *POMGNT1*, *LARGE*, *FKTN* and *FKRP*. With the exception of *pomgnt1*, analyses of morpholino-induced knockdown in zebrafish confirm that the orthologous enzymes function similarly in zebrafish and humans (Thornhill et al., 2008; Avsar-Ban et al., 2010; Kawahara et al., 2010; Lin et al., 2011). In addition, it has been reported that zebrafish *fukutin* (*fktn*) or *fukutin-related protein* (*fkrp*) morphants show endoplasmic reticulum stress and activation of the unfolded protein response. Furthermore, vacuoles of notochord cells in these morphants contain protein accumulations that are marked by immunoreactivity with IIH6 (Lin et al., 2011), a monoclonal antibody detecting glycosylated α-dystroglycan (Ervasti and Campbell, 1993). Similar accumulations of low-molecular-weight protein complexes were also detected in α-dystroglycan-deficient zebrafish, indicating

that protein secretion in dystroglycanopathies is affected beyond glycosylation of α-dystroglycan (Lin et al., 2011).

Recently, mutations in the *ISPD* gene have been identified as the second most common cause of Walker-Warburg syndrome, a type of congenital MD (Roscioli et al., 2012). Morpholinos targeting *ispd* in zebrafish induced a phenotype that recapitulates the features of Walker-Warburg syndrome, confirming that the detected mutations in *ISPD* were eliciting the human condition. In addition, a cooperative interaction between *ispd*, *fktn* and *fkrp* in glycosylating α-dystroglycan was identified in zebrafish, demonstrating that functions previously unidentified in vertebrates can be elucidated in zebrafish (Roscioli et al., 2012). This study reinforces the usefulness of zebrafish studies for providing functional data for mutations identified in muscle disease patient cohorts.

Modelling congenital MD
The heterotrimeric laminins are major structural components of the basal lamina and are formed by three distinct chains: one α-, one β- and one γ-chain. Laminin-2 is the major muscle isoform of laminin, and is formed by a combination of α2-, β1- and γ1-chains. It can bind to either α7β1 integrin or, through α-dystroglycan, the DGC, thereby representing a core component of the two major complexes that connect the actin cytoskeleton of the myofibres to the ECM (Fig. 1). Humans with mutations in laminin α2 can acquire severe congenital MD with

merosin deficiency (MDC1A; OMIM 607855) (Helbling-Leclerc et al., 1995). Although various mouse models of laminin α2 deficiency have provided valuable insights into disease development, the exact molecular basis of MDC1A pathology is not fully understood (Gawlik and Durbeej, 2011). Analysis of the zebrafish mutant *candyfloss* (*caf*), which carries a null allele of *lama2*, has revealed that myofibre detachment is triggered by mechanical load of the fibres rather than an intrinsic defect in cell signalling (Hall et al., 2007). Consistent with laminin-2 being located in the basal lamina outside the fibre, the sarcolemma of detaching fibres is not compromised, and detached fibres subsequently undergo apoptosis rather than necrosis, unlike the necrosis seen in DMD, where the sarcolemma is compromised (Fig. 1) (Hall et al., 2007). Importantly, muscle formation and myoblast fusion are normal in *caf* mutants, and primary motor neurons appear unaffected (Hall et al., 2007), further suggesting that mechanical load on the myofibre is the primary cause of muscle pathology in MDC1A.

Interestingly, the zebrafish mutant *softy* (*sof*), which carries a missense mutation in the *lamb2* gene, also shows myofibre detachment at the myotendinous junction (Jacoby et al., 2009). In contrast to the *dmd* and *caf* mutants, detached myofibres remain viable and form ectopic myosepta in *sof* mutants, exposing a newly identified pathway that could be targeted in therapy for MDs (Jacoby et al., 2009). Although mutations in *LAMB2* have so far only been associated with neuromuscular junction defects in individuals suffering from congenital myasthenic syndrome (OMIM 608931) (Maselli et al., 2009), the dystrophic phenotype of *sof* mutants indicates that *LAMB2* is a candidate gene for genetically uncharacterized human MDs.

Another laminin-2 receptor in skeletal muscle is the heterodimeric α7β1 integrin, which, together with integrin-linked kinase (ILK), forms part of the integrin-ECM adhesion complex that structurally links the actin cytoskeleton and the ECM (Fig. 1). In rare cases, mutations in α7 integrin (*ITGA7*) lead to congenital MD with integrin α7 deficiency (OMIM 613204), in which patients exhibit delayed motor milestones (Hayashi et al., 1998). In zebrafish, morpholino-based knockdown of *itga7* induces a dystrophic phenotype similar to the

lost-contact (*loc*) mutant, which carries an *ilk* null allele that leads to loss of integrin function (Knöll et al., 2007; Postel et al., 2008). In both *loc* mutants and *itga7* morphants, myofibres detach from the myosepta, leading to progressive paralysis (Postel et al., 2008). Importantly, the zebrafish-based study of Knöll and colleagues demonstrates an epistatic regulation between *ilk* and *lama4*, which led to the identification of previously unknown *ILK* and *LAMA4* mutations in individuals with dilated cardiomyopathy (Knöll et al., 2007). Also noteworthy is the report that knockdown of the integrin binding partner kindlin-2 also leads to skeletal muscle damage in zebrafish (Dowling et al., 2008). Although no humans have been identified with mutations in the orthologous *KIND2* to date, it might be a candidate gene for MD.

Modelling limb-girdle MD

To date, 25 types of limb-girdle MDs caused by mutations in at least 21 different genes have been catalogued (Kaplan, 2011). One of these genes encodes δ-sarcoglycan, a component of the DGC (Fig. 1), and morpholino-based studies in zebrafish have revealed that it is also required for muscle integrity in zebrafish (Guyon et al., 2005; Cheng et al., 2006). Another of these genes encodes dysferlin (DYSF) (Bashir et al., 1998; Liu et al., 1998), a key player in the repair of sarcolemmal lesions, which are frequently provoked in myofibres by mechanical stress (Bansal et al., 2003). Knockdown of *dysf* in zebrafish results in muscle abnormalities (Kawahara et al., 2011a) and, in a recent study, this model was used to shed new light on the exact sequential steps of sarcolemmal repair (Roostalu and Strähle, 2012). Taking advantage of the ability to undertake subcellular high-resolution imaging in living muscle in zebrafish, the authors visualized at the lesion site the formation of a repair patch that is independently assembled by the cytoplasmic annexin A6 (Anxa6) and vesicles enriched with dysferlin. Subsequently, Anxa2a is recruited to the repair patch, followed by Anxa1a, which covers the sarcolemmal lesion (Roostalu and Strähle, 2012). These studies provide a new understanding of the mechanism directed by dysferlin during the patch repair process at the damaged sarcolemma of a muscle cell, and highlight the potential to observe dynamic molecular interactions in live zebrafish.

Modelling Ullrich congenital MD and Bethlem myopathy

In addition to defective sarcolemma-spanning complexes, deficiencies in the ECM (which provides structural support to myofibres) can also cause MDs. Mutations in COL6A1, COL6A2 or COL6A3, which together form the ECM protein collagen VI, can elicit Ullrich congenital MD (UCMD; OMIM 254090) or Bethlem myopathy (BM; OMIM 158810) (Jöbsis et al., 1996; Camacho Vanegas et al., 2001). Individuals with UCMD typically suffer from severe muscle weakness with excessive joint hypermobility and contractures, whereas those with the milder BM usually have slow, progressive muscle weakness. Although *Col6a1* loss-of-function mice only have a mild myopathy, reminiscent of BM (Bonaldo et al., 1998), both diseases have been modelled by using two distinct morpholinos in zebrafish (Telfer et al., 2010). Morpholinos targeting exon 9 of the orthologous zebrafish *col6a1* led to a severe UCMD-like phenotype with early onset of motor deficits, whereas targeting of exon 13 induced mild myopathy with late onset of motor deficits, reminiscent of BM (Telfer et al., 2010). The ameliorative effect of the proton pump modifier cyclosporine A, described in *col6a1*-deficient mice (Irwin et al., 2003), was confirmed in UCMD-like zebrafish. Interestingly, although cyclosporine A improved mitochondrial appearance and decreased apoptosis, sarcolemmal integrity was not ameliorated in UCMD-like zebrafish (Telfer et al., 2010). As noted for dystrophin-deficient zebrafish above (Berger et al., 2010), severe human MDs such as UCMD or DMD are more closely modelled in zebrafish than in mice. Moreover, these zebrafish models are powerful and highly efficient for evaluating therapeutic strategies.

Concluding remarks

The results discussed above illustrate the fact that many common MDs have been successfully modelled in zebrafish. The classical ways of generating disease models in zebrafish via forward genetics based on random mutagenesis or morpholino suppression have been an important adjunct to disease gene discovery and validation. However, the lack of gene targeting has been a bottleneck in the zebrafish field. The non-directional nature of these mutations, however, enables the discovery of hypomorphic or dominant-negative alleles, which are usually induced by missense mutations. In principle, such mutations

can also be re-engineered by targeted recombination – but which mutation elicits a phenotype is difficult to predict. The random mutation approach can also lead to the identification of gene functions that have not yet been associated with a certain disease condition. Nonetheless, zebrafish are at the beginning of a new age of genome manipulation: direct gene disruption is now possible with zinc-finger-nuclease and TALEN technology (Sander et al., 2011). The plummeting costs of sequencing have also enabled the identification of mutations based on the sequencing of whole DNA libraries of mutagenized genomes. This approach has sparked large-scale ventures such as the Zebrafish Mutation Project, which aims to generate a knockout allele for every zebrafish protein-coding gene (http://www.sanger.ac.uk/Projects/D_rerio/zmp). These approaches will undoubtedly increase the ability to generate human disease models in zebrafish.

Another major scientific stronghold of the fish is the ease with which dynamic cell processes can be visualized in vivo and in real time with time-lapse movies. The ability to image living muscle tissue in a disease context and to examine pathology onset in real time is a significant advantage of the zebrafish system. Myofibre detachment has been documented in dystrophin-deficient live zebrafish (Berger et al., 2010) and even subcellular processes such as the repair of myofibre lesions can be studied in this way (Roostalu and Strähle, 2012). Owing to the cost-effective breeding and husbandry of the zebrafish, small-molecule screens can be performed against dystrophic phenotypes at relatively high throughput to identify and further analyze lead drugs (Berger and Currie, 2007; Kawahara et al., 2011b). In future, more screens will be performed to identify additional compounds that might one day become effective medications to treat MD in humans.

In summary, although zebrafish are phylogenetically more distant from humans than mammalian model organisms, the main functions of the orthologous muscle genes reviewed above are conserved between zebrafish and human. Together with the unique set of tools available, the zebrafish system therefore provides valuable models for studying human MD.

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