A Drosophila model for Duchenne muscular dystrophy

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CHAPTER 7

General Discussion

1. Potential formation of complexes between the *Drosophila* DGC proteins

The genes encoding the majority of the mammalian DGC proteins are also present in conserved form in the *Drosophila* genome. The *Drosophila* DGC transcripts are found in a variety of tissues and cell types, most predominantly in muscle and CNS and they show patterns of tissue-specific mRNA expression similar to that seen of their homologs in mice and humans (Chapter 2 of this thesis).

As in mammals, the mRNAs are often co-expressed in particular tissues, suggesting that their encoded proteins could form DGC-like complexes there. We have analyzed the Dystrophin and Dystrobrevin protein expression patterns and find that they localize to similar domains in the muscle and the CNS. Additionally, Dystrobrevin expression disappears in BL7663 Dystrophin deficient embryos at the muscle attachment sites and in larvae in the ventral nerve cord and eye-antennal disc. Analysis of Dystrobrevin expression in the $dys^{DLP2\ E6}$ mutant, revealed that wild type Dystrobrevin expression at the embryonic muscle attachment sites and in the third instar larval eye-antennal disc depends specifically on the presence of DLP2. These results indicate that the *Drosophila* Dystrophin and Dystrobrevin proteins likely form a complex similar to that seen in vertebrates. In contrast, Dystrobrevin expression in the optic tectum is not altered by the absence of Dystrophin, even though both Dystrobrevin and the Dystrophin Dp186 isoform are expressed there, suggesting that Dystrobrevin is localized by some other mechanism in that tissue. It will be interesting to see where the proteins of the other *Drosophila* DGC members are expressed and if their localization also depends on Dystrophin or Dystrobrevin. Dystrobrevin mutants are not yet available, but future analysis of the expression patterns of the other DGC members as well as Dystrophin in Dystrobrevin mutants could prove informative.

2. The roles of DLP2 and Dp186 at the synapse

We have generated mutants that lack the muscle specific DLP2 long isoform or the CNS specific short isoform Dp186 (Chapter 3 and van der Plas *et al.*, 2006; Chapter 4). Analysis of these mutants have revealed apparently similar postsynaptic roles in the regulation of presynaptic neurotransmitter release, though their sites of action are at the NMJ and central nervous system interneuronal synapses, respectively (Figure 1A). The absence of the Dystrophin DLP2 and Dp186 isoforms or reduction of their expression levels results in an increased amount of evoked neurotransmitter release, which hyperdepolarizes the muscle or the receiving motoneuron. A similar effect is seen in the musculature of Dystrophin deficient *C. elegans* lines (Bessou *et al.*, 1998; Gieseler *et al.*, 2001), although the increased neurotransmission in the worm is not a consequence of increased release, but is due to the increased sensitivity to acetylcholine and reduced clearing of acetylcholine from the synaptic cleft, subsequent to delocalisation of an acetylcholine transporter (Ségalat, 2002; Carre-Pierrat *et al.*, 2006; Kim *et al.*, 2004). In mice, defects in synaptic transmission are not easily determined because aspecific effects caused by the muscle degeneration make clear readings technically difficult. However, as we observe at the DLP2-deficient *Drosophila* NMJ, mEJPs are unaffected at the *mdx* mouse NMJ (Lyons & Slater, 1991).

While the functions of DLP2 and Dp186 appear similar, their protein structures differ with respect to the presence of an actin-binding domain. Dp186 lacks an actin-binding domain, but performs an apparently similar role in regulation of neurotransmitter release at interneuronal synapses as DLP2 at the NMJ. Therefore, the actin-binding domain of DLP2, which links the DGC to the actin cytoskeleton, might not be required for this function. Future experiments can address this question by testing whether expression of DLP2 without an actin-binding domain can rescue the mutant phenotype. The absence of the actin-binding

domain in Dystrophin was originally correlated to the muscle degeneration in mice, although our results indicate that yet another Dystrophin isoform without an actin-binding domain is required for muscle integrity in *Drosophila*. In view of this, it would seem that the DLP2 Dystrophin isoform does not have a similar function in muscle as the Dp427 isoform in mammals, but that DLP2 function more closely resembles that of Utrophin, which is also primarily located at the NMJ (Weir *et al.*, 2002). However, removing Utrophin leads to NMJ abnormalities, characterized by a reduction in AChR clustering and synaptic folding, while removing DLP2 has a predominantly functional effect in increased vesicle release, which correlates with a presynaptic increase in the number of T-bars per active zone. Glutamate receptor clustering and postsynaptic ultrastructure remains unchanged in $dys^{DLP2\ E6}$ mutants (van der Plas *et al.*, 2006; Chapter 3 of this thesis).

Which proteins interact with DLP2 or Dp186 at the synapse? We have shown that the increase in neurotransmitter that results in the absence of DLP2 at the NMJ is dependent on the presence of the BMP receptor Wishful thinking (Wit; van der Plas *et al.*, 2006 and Chapter 4). However, we also show that the downstream effector, PMad, of the BMP signaling pathway is unaltered in DLP2 mutants, suggesting an alternate pathway is involved, downstream of Wit. As mentioned in the introduction of this thesis, the BMP pathway could play a role in retrograde signaling via a PMad independent route through for instance LIMK1, or it can be only a permissive pathway.

A likely candidate for interaction with Dystrophin at the synapse is CaMKII, which has been reported to be involved in retrograde signaling (see Introduction). *Drosophila* larvae expressing a dominant-negative CaMKII inhibitor show increases in quantal content accompanied by an increase in T-bar numbers (Haghighi *et al.*, 2003), similar to those observed in $dys^{DLP2\ E6}$ larvae. However, despite the similarities in phenotypes between larvae with reduced levels of CaMKII and the $dys^{DLP2\ E6}$ mutant, we have not yet been able to uncover a genetic interaction between *dystrophin* and *CaMKII*.

Synaptotagmin 4 (Syt4), which is present on postsynaptic vesicles, might function as a Ca^{2+} sensor for release of the retrograde signal, but is a less likely candidate for an interaction with Dystrophin since Syt4 mutants show defects in NMJ formation (Yoshihara *et al.*, 2005), which are not seen in $dys^{DLP2\ E6}$ mutants. Since Syt4 is a component of exocytotic vesicles in the muscle, it is not likely to be a part of the retrograde signal in itself, but could be part of the exocytotic machinery required to release the retrograde signal. Lack of Syt4 may therefore not only impede the retrograde signal, but also the release of proteins important for maintenance of the synapse, e.g. Gbb or other trophic factors.

The mammalian DGC has been shown to bind nNOS via the Syntrophin PDZ domain (Brenman *et al.*, 1996). Mice bearing mutations in Dystrobrevin or Syntrophin, which have displaced nNOS, or nNOS mutants themselves show a reduction in AChR clustering and synaptic folding, indicating possible roles for nNOS in NMJ maturation (Grady *et al.*, 2000; Adams *et al.*, 2000). It is likely that nNOS also binds to the DGC in *Drosophila* via the PDZ domain in Syntrophin, but this remains to be investigated. nNOS creates NO, which has been shown to induce vesicle release via cGMP in *Drosophila* (Wildemann & Bicker, 1999a; Wildemann & Bicker, 1999b) and, since it easily diffuses between different tissues, is a candidate retrograde signal.

What proteins are targeted at the presynaptic side of the synapse? Bruchpilot, which shows homology to human active zone protein ELKS/CAST/ERC, is likely to be involved, since it is a component of T-bars (Wagh *et al.*, 2006; Kittel *et al.*, 2006). An increase in T-bar number likely requires an increase in Bruchpilot expression. It is possible that the increase in T-bars in itself is sufficient to cause the increase in the probability of release seen in Dystrophin mutants. Having more active release sites would facilitate an increase in the total number of

vesicles released at the same time. Ongoing experiments are directed at evaluating whether or not quantal content and T-bar numbers increase at the DLP2- and Brp-deficient double mutant NMJ. We cannot rule out the possibility of other changes in the release mechanism at these synapses, such as vesicle recruitment to the membrane or the exocytotic machinery, although the change would have to be [Ca^{2+}] independent (see Chapter 3, Fig. 5A).

It is tantalizing to link the defect in neurotransmitter release regulation in Dystrophin mutants to the mental retardation that is found in approximately one third of the DMD patients (average IQ is 85; Anderson *et al.*, 2002). In both mice and flies, mutations affecting proteins involved in synaptic function are often found to have defects in memory or other cognitive functions such as spatial recognition. Mental retardation in DMD patients has been linked to mutations in the carboxyterminal domain of the protein (Anderson *et al.*, 2002; Moizard *et al.*, 2000), indicating the possible involvement of one or more of the shorter Dystrophin isoforms, which are predominantly expressed in brain tissues. Lack of Dystrophin also leads to morphological defects in brains of Dystrophin deficient mice, defects in passive avoidance behaviour and impaired short term memory (Anderson *et al.*, 2002). The behavioral consequences of increased levels of neurotransmitter release at interneuronal synapses in the *Drosophila* Dp186 mutant CNS have yet to be analyzed. Other roles for Dp186 may be uncovered in the eye, since Dp186 protein is expressed in the optic tectum and in the ommatidium, the repeating functional unit of the compound eye. DMD patients show EEG abnormalities and an increased prevalence of red-green color vision impairment, indicating likely roles for human Dystrophin isoforms in synaptic connectivity within the ocular system (Anderson *et al.*, 2002; Costa et al., 2007).

3. Roles of Dp117 and Dystrobrevin in muscle degeneration, survival and wing formation

In this thesis, it is shown that absence of the Dystrophin Dp117 isoform, as well as absence of Dystrobrevin, has effects on the integrity of the third larval instar body wall musculature (Figure 1B; Chapters 5 and 6 of this thesis). Recently, a role for Dystrophin and Dystroglycan in maintaining the aging adult fly musculature has been reported (Shcherbata *et al.*, 2007), but no distinction was made in this study between the different isoforms. It is as yet not clear what molecular mechanism causes muscle degeneration in these larvae and flies from both studies.

Based on studies of DMD patients and mice bearing mutations in the genes encoding some of the DGC proteins, several models of DGC function in striated muscle have been proposed (for review see Petrof *et al.*, 2002; Deconinck & Dan, 2007). I will here discuss the following models, 1) a mechanical/structural role, 2) a role in Ca^{2+} homeostasis, 3) a role in NO regulation, and 4) a role as a scaffold for signaling pathways.

3.1 A mechanical/structural role

Since the DGC links the extracellular matrix (ECM) to the actin cytoskeleton, the most obvious potential function for the DGC is stabilization of the sarcolemma during contraction and preventing it from rupturing. This is supported by the many studies that show that activity of the muscle in DMD patients and *mdx* mice accelerates the dystrophic phenotype. The presence of an actin-binding domain in Dystrophin has been shown to be essential for protection against muscle degeneration in mice (Judge *et al.*, 2006). However, reports of muscle degeneration in mice lacking Dystrobrevin (Grady *et al.*, 1999) or Sarcospan (Peter *et al.*, 2007) demonstrate that muscle degeneration still occurs in the presence of sarcolemmally-localized Dystrophin with a functional actin-binding domain and when sarcolemmal integrity is still intact. This suggests that the actin-binding domain in mammals might very well be important for sarcolemmal integrity, but that the muscle degeneration is

not necessarily caused by membrane rupture or leakage. Membrane damage and muscle degeneration may therefore be contemporaneous unlinked effects. Membrane damage arising from the disruption of the ECM-cytoskeleton link in the absence of Dystrophin may aggravate the dystrophy.

Further evidence for the possibly unlinked, but additive effects of sarcolemmal damage and muscle degeneration comes from transgenic expression of either the Dp260 or Dp116 Dystrophin isoforms in the muscles of *mdx* mice. Warner *et al.* (2002) examined the effects of ectopic expression in *mdx* muscles of the Dp260 Dystrophin isoform, which lacks the N-terminal actin-binding domain, but retains the spectrin repeat actin-binding domain. They show that Dp260 does not prevent the muscle degeneration, but does rescue the sarcolemmal damage, supporting the theory that membrane disruption and the absence of an actin-binding domain is not required for the onset of muscular dystrophy. Judge *et al.* (2006) attempted to rescue the muscle degeneration in *mdx* mice by expressing the non-muscle isoform Dp116, which lacks both actin-binding domains, and found that muscle degeneration is more severe than in *mdx* muscle and sarcolemmal damage is still present, suggesting that the actin-binding domain is required for membrane stability.

Our results on the recently identified Dp117 Dystrophin isoform in *Drosophila* are consistent with the theory that sarcolemmal disruption is not required for the onset of muscular dystrophy. Reduction of pan-Dystrophin causes activity and/or time-dependent muscle degeneration in both larvae and adults without apparent effects on sarcolemmal integrity (Chapter 5 of this thesis). These results suggest that membrane damage due to disruption of the link between the actin cytoskeleton and the ECM does not necessarily underlie muscle degeneration.

Another mechanical/structural explanation for why muscle degeneration occurs in the absence of Dystrophin is the impairment of the structural link between the costameres and the DGC. The absence of Dystrophin results in a disorganized costameric lattice and disruption of sarcolemmal integrity (Cohn & Campbell, 2000; Blake *et al.*, 2002). Both Dystrophin and Dystrobrevin have been shown to bind to proteins associated with costameres, such as γ-actin, synemin and syncoilin, thereby linking the sarcolemma to the costameres surrounding the Z-disk.

Since Dystrobrevin null mice display muscle degeneration with an intact sarcolemma, the hypothesis was originally proposed that Dystrobrevin assumes a signaling role in the onset of muscular dystrophy (Ervasti, 2003). However, defects in cell signaling in the absence of Dystrobrevin have not yet been detected. Dystrobrevin has been found to bind a number of intermediate filament proteins, such as syncoilin and synemin (Mizuno *et al.*, 2001; Poon *et al.*, 2002), which in turn bind to desmin. Desmin has been shown to constitute one of the links between the Z-disk and the sarcolemma (Ervasti, 2003). Loss of desmin leads to a weak muscle phenotype with impaired lateral force transmission. Although membrane integrity has not been closely examined in desmin mutant muscles, the absence of force drop with eccentric contraction suggests that their sarcolemma remains largely intact (Ervasti, 2003). Therefore, disruption of the link between the costameres and the sarcolemma results in impaired sarcolemmal function. This disruption could lead to the onset of muscle degeneration. Dystrobrevin, similar to desmin, may have a role in lateral force transmission by coupling the intermediate filament cytoskeleton to the DGC. Dystrobrevin thus possibly serves a mechanical or structural function in the link between the Z-disk and the sarcolemma. Similarly to the *mdx* and *dystrobrevin* mutant mice, muscle degeneration in Drosophila larvae with reduced expression of Dystrophin Dp117 is accompanied by a disruption of the sarcomeric Z-band (Chapter 5 of this thesis; van der Plas *et al.*, 2007). The sarcomeric structure of larval muscles with reduced Dystrobrevin expression has not yet been closely examined, but is likely disrupted as well.

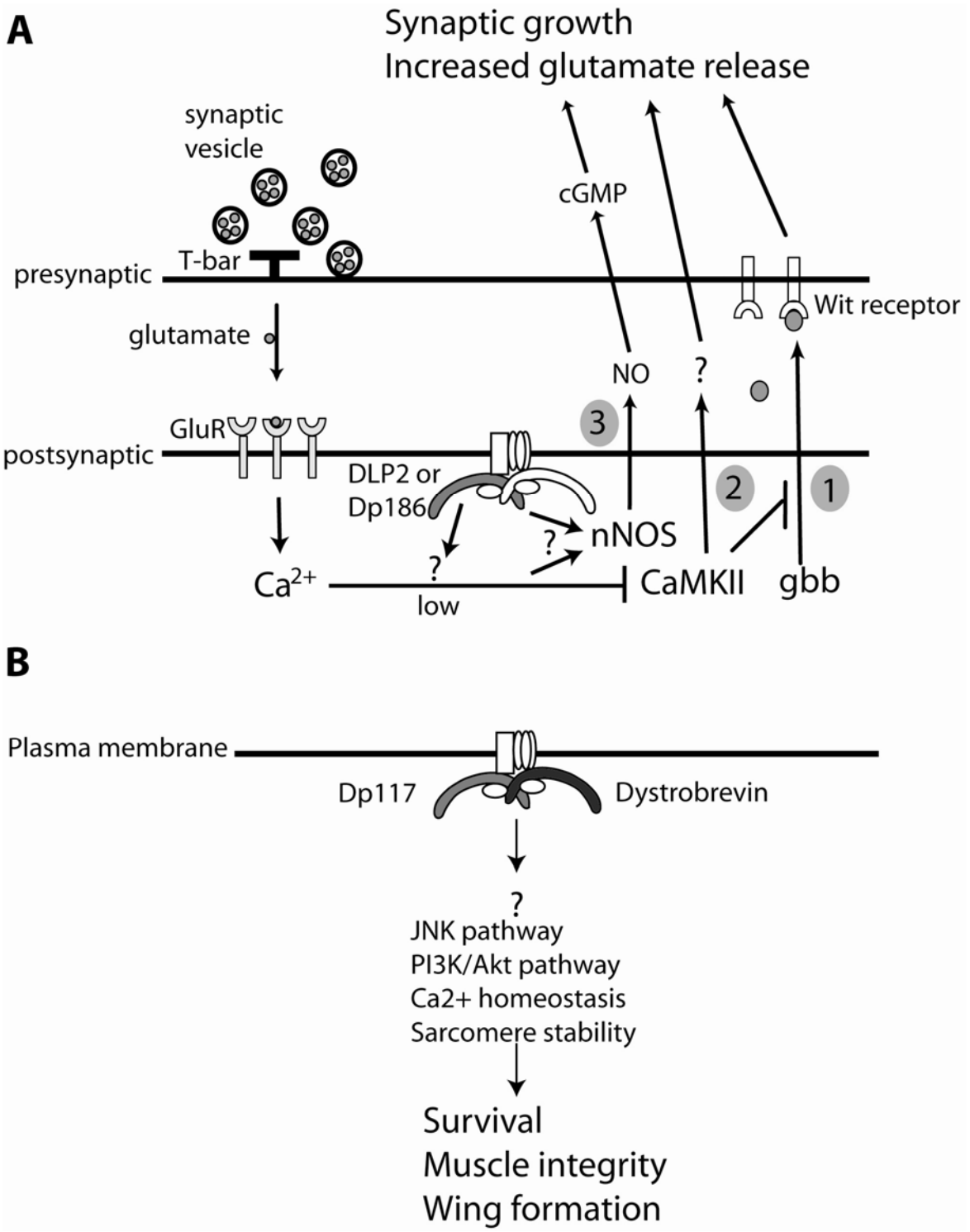

Figure 1: A summary model for the different functions of Dystrophin isoforms in *Drosophila melanogaster*. (**A**) A model for the function of DLP2 and Dp186 at the synapse. The DGC most likely anchors proteins (CaMKII or a CaMKII activating protein) at the sarcolemma, which respond to low Ca^{2+} levels in the cytosol resulting in CaMKII deactivation. This can lead to 1) inhibition of the BMP pathway by decreasing the amount of Gbb released, or 2) the release of another unknown factor which is taken up by the presynaptic side, or alternatively 3) nNOS is activated and produces NO, which causes the production of cGMP in the presynaptic side. Improper activation or deactivation of one or a combination of these pathways results in increased vesicle release in the absence of Dystrophin. (**B**) A hypothetical model for the possible functions of Dp117 and Dystrobrevin in survival, muscle maintenance and wing formation. Absence of the DGC could lead to activation of apoptotic pathways, such as the JNK pathway, or it could lead to a growth defect via PI3K/Akt members of the insulin pathway. Other hypotheses for how muscle degeneration begins, include the disruption of Ca^{2+} homeostasis in the absence of the DGC or the elimination of a mechanical link between the sarcolemma and the sarcomeres via the costameric lattice.

It is not yet known how disruption of the sarcomeres leads to muscle degeneration. The mechanism leading to degeneration in *dystrophin* or *dystrobrevin* mutant muscles could be similar to that seen in patients with Limb girdle muscular dystrophy type 2A (LGMD2A), which is caused by mutations in calpain-3 leading to disruption of the sarcomeres. It is possible that eliminating the link between the sarcolemma and the costameric lattice results in destabilization of the sarcomeres due to calpain delocalization or deactivation (reviewed in Duguez *et al.*, 2006; Spence *et al.*, 2002). Calpain expression in affected muscles of larvae with reduced levels of Dp117 or Dystrobrevin has not yet been determined.

3.2 Ca^{2+} homeostasis

A second potentially important mechanism in the onset of muscular dystrophy is disruption of Ca^{2+} homeostasis (Alderton & Steinhardt, 2000; Deconinck & Dan, 2007). Internal Ca^{2+} levels are increased in Dystrophic muscle due to sarcolemmal damage linked to muscle activity and abnormally active calcium leak channels. The elevated Ca^{2+} levels result in calcium-dependent proteolysis through activation of calpain. It is hypothesized that Dystrophin, through the DGC, assembles signaling pathways involved in Ca^{2+} homeostasis, or that the DGC regulates the cytoskeleton, which in turn regulates the activity of ion channels in the sarcolemma as well as the sarcoplasmic reticulum. However, reports are contradictory and the use of myotubes versus muscle fibers as well as the particular fiber type and activity of the muscle influence experimental outcomes (see Constantin *et al.*, 2006 for review).

Inactivity of the muscle seems to slow down the degeneration process as determined from studies of hind-limb immobilization in *mdx* mice and one Becker Muscular Dystrophy patient with spina bifida (Kimura *et al.*, 2006; Mokhtarian *et al.*, 1999). The activity-dependent degeneration in *mdx* muscles suggests that membrane damage causes increased Ca^{2+} levels, and possibly suggests an involvement of mechanosensitive membrane channels (Deconinck & Dan, 2007). Channels whose mutations are correlated with the inappropriate entry of Ca^{2+} include the stress-activated-channels (SACs) (Yeung *et al.*, 2005) and store-operated-channels (SOCs) (Pan *et al.*, 2002). In *C. elegans*, the lack of Dystrophin protein alone is not sufficient to cause muscle degeneration. However, double mutants for Dystrophin and the Egl-19 Ca^{2+} channel do display muscle degeneration, indicating that Ca^{2+} homeostasis plays a crucial role in the onset of muscle degeneration in worms (Mariol and Ségalat, 2001).
In addition to the increase in Ca^{2+} due to leakage, it is shown that *mdx*-fibers have a reduced capability to buffer Ca^{2+} levels due to decreased expression of sarcalumenin (Dowling *et al.*, 2004). Mitochondria also play roles in Ca^{2+} homeostasis in the muscle. Ca^{2+} level peaks in the cytosol are sensed and amplified in mitochondria (Robert *et al.*, 2001). In *mdx* muscle, this mitochondrial response was found to be increased, which may possibly trigger the apoptotic mechanisms that precede necrosis of the degenerating muscle (Gailly, 2002).

Electrophysiological analysis of dystrophic muscle fibers has given insight into the mechanism of muscle degeneration. Drastic changes in the resting potentials of Dystrophin-deficient skeletal muscle fibers, which might be expected if Dystrophin deficiency influences the gating of channels active at rest, were not observed (Allard, 2006). The outcomes of experiments that test membrane leakage at resting potentials and excitability of *mdx* fibers are mostly conflicting. The only apparent difference in channel function at the sarcolemma between normal and *mdx* muscles seems to be the increased open probability of sarcolemmal cation channels active during muscle rest (Allard, 2006).

As shown from the studies mentioned above, much is still uncertain pertaining to the involvement of Ca^{2+} in the onset of muscular dystrophy. In order to further characterize the possible involvement of Ca^{2+}, we are currently exploring options for elucidating the role of Ca^{2+} in muscle degeneration in third instar larvae with reduced expression levels of Dp117 and/or Dystrobrevin. The internal Ca^{2+} levels have not yet been investigated for muscles with reduced expression of Dp117 or Dystrobrevin. The intact sarcolemma in affected muscles in

these larvae excludes the possibility of increased Ca^{2+} levels due to membrane ruptures. Thus if internal Ca^{2+} levels are found to be different from wild type, the change would likely be caused by increased Ca^{2+} entry through Ca^{2+} channels in either the sarcolemma (eg. Ca α1D) or the sarcomeric reticulum (eg. the ryanodine receptor), or alternatively, by reduced clearing of cytosolic Ca^{2+} (eg. into the sarcomeric reticulum by SERCA).

3.3 NO regulation

A third theory for the mechanism of muscle degeneration involves the direct or indirect misregulation of NO in dystrophic muscle. NO has been reported to play a role in the onset of muscle degeneration in *mdx* mice (Wehling *et al.*, 2001; Tidball *et al.*, 1999; Chaubourt *et al.*, 2002). NO may function as a protection against muscle degeneration in muscle, since NO can function as a vasodilator, protecting muscles from hypoxia. The neuronal NOS isoform, nNOS, is displaced from the sarcolemma of cardiac muscle cells in the absence of Dystrophin, which likely precludes the production of NO and results in hypoxic muscle damage. In skeletal muscle, the function of NO may be more complex. Delocalization of nNOS from the sarcolemma is hypothesized to cause the inappropriate formation of free radicals, which damage the muscle cell. Although restoration of NO levels to normal in *mdx* muscle ameliorates degeneration (Wehling *et al.*, 2001), this may be due to the secondary effects of NO, e.g. increased expression levels of the costameric proteins talin and vinculin, as well as Utrophin (Tidball *et al.*, 1999; Chaubourt *et al.*, 2002). We have yet to investigate the role of NO in the onset of muscle degeneration in third instar larvae with reduced expression levels of Dp117 or Dystrobrevin.

3.4 Scaffold for signaling pathways

A fourth hypothesis concerning the onset of muscle degeneration in the absence of Dystrophin is that Dystrophin with the other DGC proteins forms a scaffold for signaling pathways. Absence of Dystrophin disrupts the formation of this scaffold, resulting in misregulation of signaling pathways leading to e.g. apoptosis, which is thought to precede the necrosis in *mdx* muscle (Sandri *et al.*, 1998; Tidball *et al.*, 1995) or other defects. Defects in signaling pathways could underlie the lethality and muscle degeneration we have described in larvae with decreased Dp117 expression.

We show that 2xRNAi-Dp117-24B larvae stop feeding between second and third larval instar stage and that they are transparent due to an apparent lack of fat bodies. It is unclear why they stop feeding and whether this is a direct cause for or a result of the subsequent lethality, since onset of necrosis in the intestinal tract could induce the larvae to stop feeding. The 2xRNAi-Dp117-24B larvae resemble those mutant for the *Drosophila* class I_A PI3-kinase, Dp110 (Weinkove *et al.*, 1999). PI3K is involved in the insulin signaling pathway, which regulates cell growth as well as cell number (for review see Edgar, 2006). Dystrophin has been linked to Akt signaling in the hypertrophic response in DMD and LGMD muscles (Peter & Crosbie, 2006) as well as in the mechanisms underlying cachexia, skeletal muscle atrophy, which is a common feature of cancer (Acharyya *et al.*, 2005). In *Drosophila*, Shcherbata *et al.* (2007) recently proposed a link between Dystroglycan and the insulin receptor pathway in axon guidance in the *Drosophila* brain. Furthermore, overexpression of insulin signaling pathway members dS6K and dAkt results in curved up wings due to a difference in cell size between the dorsal and ventral wing blade (Rintelen, 2001). A curved up wing phenotype also results from reduced expression of all Dystrophin isoforms or Dp117 alone (Chapter 6) and may therefore reflect underlying cell growth deficits similar as in dS6K and dAkt wings.

A curved up wing phenotype could alternatively be caused by differences in cell number between the dorsal and the ventral wing blade. Cell number can be regulated by the BMP homolog Dpp. Dpp deficient cells are excluded from the wing blade, but do not die, and show activitation of the JNK pathway, which can lead to apoptosis (Gibson & Perrimon, 2005;

Shen & Dahmann, 2005; Adachi-Yamada *et al.*, 1999). As yet, we have no indication of apoptosis taking place when expression levels of Dp117 are reduced.
Additionally, DLP2 mutant wings show a failure of the posterior and anterior cross veins to properly form. Such defects are also seen in mutants for crossveinless-2 (cv-2), which was recently shown to be a regulating protein of Dpp (Conley *et al.*, 2000). It is possible that, like cv-2, Dystrophin plays a role in maintaining or regulating BMP signaling.

Studies in both mice and *Drosophila* indicate a link between the DGC and the Egfr signaling pathway or its downstream target, ERK-MAP (extracellular signal-regulated kinase-mitogen-activated protein). γ-Sarcoglycan deficiency in mice seems to activate ERK (Barton, 2006). Similarly, reduction of β-Sarcoglycan expression levels induces Egfr signaling activation in the *Drosophila* eye (Hashimoto & Yamaguchi, 2006). In mice, Dystroglycan interacts with several components of the ERK-MAP kinase cascade and modulates ERK activity in response to the binding of integrin to laminin (Spence *et al.*, 2004). In the *Drosophila* oocyte, Egfr signaling downregulates Dystroglycan levels for proper localization of posterior polarity (Poulton & Deng, 2006). Thus, several members of the *Drosophila* DGC have been shown to interact with the Egfr signaling pathway or one of its downstream effectors, ERK. However, it is not known if this pathway is involved in muscle degeneration.

It is difficult to separate the principal cause of muscular dystrophy from the secondary effects that arise once the muscle is damaged. At this point it is therefore unclear which of the above named complications first arises in the disease process. Furthermore, it is possible that several or even all of these mechanisms act together. Analysis of early effects in the disease progression could help distinguish between the possible mechanisms for muscle degeneration. With our work on the function of Dystrophin in the development of *Drosophila*, we have uncovered novel Dystrophin functions in the retrograde regulation of neurotransmitter release at the synapse. Furthermore, we have shown that Dystrophin in the fruit fly is important for survival during development, as well as for the survival of individual muscle cells. By taking advantage of the amenability of *Drosophila* for genetic analysis and its rapid lifecycle, these phenotypes will be used in genetic screens to further discover other molecules involved in these processes and thereby increase the understanding of the pathogenesis of muscular dystrophies.

4. References

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