Utrophin upregulation for treating Duchenne or Becker muscular dystrophy: how close are we?

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Duchenne muscular dystrophy (DMD) is a severe muscle-wasting disorder for which there is currently no effective treatment. This disorder is caused by mutations or deletions in the gene encoding dystrophin that prevent expression of dystrophin at the sarcolemma. A promising pharmacological treatment for DMD aims to increase levels of utrophin, a homolog of dystrophin, in muscle fibers of affected patients to compensate for the absence of dystrophin. Here, we review recent developments in our understanding of the regulatory pathways that govern utrophin expression, and highlight studies that have used activators of these pathways to alleviate the dystrophic symptoms in DMD animal models. The results of these preclinical studies are promising and bring us closer to implementing appropriate utrophin-based drug therapies for DMD patients.

Genetic basis of Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most prevalent, genetically inherited neuromuscular disorder worldwide and affects 1 in 3500 young males [1]. DMD is caused by mutations or deletions in the gene encoding dystrophin that prevent the synthesis of full-length dystrophin molecules in skeletal muscle fibers [2,3]. Becker muscular dystrophy (BMD) is a milder form of the disease that also results from mutations in the gene encoding dystrophin; however, these mutations cause the production of reduced levels or truncated forms of the dystrophin protein. Dystrophin is present at the sarcolemma together with a group of proteins known as the dystrophin-associated protein complex (DAPC). Dystrophin binds to the intracellular cytoskeleton by associating with actin filaments at its N-terminus, whereas at its C-terminus dystrophin interacts with members of the DAPC, including β-dystroglycan. On the extracellular side of the membrane, α-dystroglycan, another member of the DAPC, binds to β-dystroglycan and also acts as a receptor for the extracellular-matrix protein laminin. Thus, dystrophin together with the DAPC forms a link between the extracellular matrix and the intracellular actin cytoskeleton, thereby providing structural integrity to muscle fibers [4,5]. Interestingly, previous studies have established that mutations in genes encoding several DAPC members result in various forms of muscular dystrophy, including congenital muscular dystrophy (caused by mutations in the α2 chain of laminin) and several limb-girdle muscular dystrophies (caused by mutations in the genes encoding sarcoglycans) [3].

The lack of dystrophin in muscles of DMD patients leads to sarcolemmal instability and disruption of the DAPC. Transient microdisruptions in the sarcolemma also occur in DMD and lead to increased levels of intracellular Ca\(^{2+}\) and subsequent alterations in Ca\(^{2+}\)-mediated signaling and calpain-mediated proteolysis. Other signaling cascades are also probably affected because the DAPC is a multifunctional signaling complex [6,7] that interacts with important additional signaling molecules such as neuronal nitric oxide synthase (nNOS) [8], Grb2 [9] and calmodulin [10]. These events cause muscle to undergo repetitive cycles of degeneration followed by muscle regeneration. Over time, the regenerative potential of dystrophic muscle fibers diminishes, resulting in progressively severe muscle necrosis and wasting. This loss of muscle mass causes patients to be confined to wheelchairs in their early teenage years and to die by the second or

Glossary

**Internal ribosome entry site (IRES):** a sequence found in the 5'UTR of viral and some cellular mRNA molecules that enables the translation-initiation machinery to assemble downstream of the 5' 7-methylguanosine cap. IRES-mediated translation in cellular mRNAs operates as a regulatory mechanism that enables increased translation initiation during situations of cellular stress where the availability of canonical translation-initiation factors might be compromised.

**Markers of the dystrophic phenotype:** in assessing the efficacy of various treatments in the mdx mouse, a widely used animal model of DMD, there are several characteristic morphological and physiological indices that can be measured, including central-nucleation index, fiber-size variability, serum creatine kinase levels, myofiber necrosis, membrane integrity and damage following lengthening contractions.

**Muscle-fiber type:** muscle-fiber type is determined by the composition of the four different myosin heavy-chain (MHC) isoforms. Slow-twitch muscle fibers express type-I myosin heavy-chain (MHC) isoforms; they use primarily oxidative metabolism and thus are more resistant to fatigue. Type-II MHC-containing fibers are classified into Ila fibers (slow twitch), which are both glycolytic and oxidative, and type IIX and IIB fibers, which are primarily glycolytic (fast twitch). Gene-expression profiles differ considerably between fast and slow muscle fibers. In this context, utrophin A is found at greater levels in extrafusal regions of slow muscle fibers compared with fast muscle fibers [48].
third decade of life as a result of cardiac or respiratory failure.

Although the molecular defect responsible for DMD was identified ~20 years ago, there is still no effective treatment available for this devastating disease other than the use of corticosteroids, which provide minimal short-term benefits. Multiple therapeutic strategies to treat DMD are currently under intense investigation in several laboratories worldwide and these include: (i) gene-based therapies that use viral vectors to either deliver functional dystrophin to muscle fibers [11] or correct for dystrophin mutations using exon-skipping strategies [12]; and (ii) cell-based therapies that involve transplantation of various types of precursor cells, such as ex vivo-manipulated muscle side population cells (a lineage of uncommitted cells), into muscle fibers [13]. There has been significant progress in the development of these strategies despite the general technical limitations in implementing cell- and gene-based therapeutics. Although such treatments are progressing towards clinical trials, several promising pharmacological strategies are also being pursued. The advantages and limitations of these three types of therapies have been the subject of several recent reviews [14,15]. A challenge for these treatments is to achieve wide distribution of the therapeutic gene product, cell or drug to the vast amount of skeletal muscle in the whole body. To this end, pharmacotherapy has the advantage of achieving systemic distribution.

Treatment with aminoglycoside antibiotics is the only drug-based therapy for DMD that aims to restore dystrophin expression; these drugs enable read-through of mutant stop codons in the gene encoding dystrophin, thus enabling full-length dystrophin to be translated [16]. Other possible pharmacological strategies are directed towards addressing the detrimental downstream effects of dystrophin deficiency, such as muscle degeneration and increased calpain-mediated proteolysis [17]. For example, to stimulate muscle regeneration, blockade of myostatin, a negative regulator of muscle growth, has been proposed as a therapeutic strategy [18], whereas reduction of calpain activity can be achieved via administration of the β2-adrenergic receptor agonist albuterol, which has already been clinically evaluated as a treatment for DMD [19]. Another promising drug-based therapy aims to compensate functionally for dystrophin by increasing endogenous levels of utrophin at the sarcolemma of dystrophic skeletal muscle fibers [20,21].

Utrophin, a suitable surrogate for dystrophin

Utrophin might be able to serve as a surrogate for dystrophin in DMD muscle fibers because there is convincing evidence for functional redundancy between these two proteins [4,22]. Indeed, utrophin shares a high degree of sequence identity with dystrophin and also associates with members of the DAPC [4,6] (Figure 1). Moreover, studies in the mdx mouse, a dystrophin negative model of DMD (Box 1), have established that the elevation of utrophin levels in dystrophic muscle fibers can restore sarcolemmal expression of DAPC members and alleviate the dystrophic pathology. This elevation can be accomplished by both germline gene transfer and somatic gene transfer of utrophin [23,24]. Together, these findings indicate that a gene-therapy approach focusing on induction of exogenously supplied utrophin to DMD muscle fibers is a plausible treatment for this disorder. Such an approach would avoid some of the adverse immune responses that are triggered by dystrophin-based gene therapy [25]. However, a more appealing approach would be to increase endogenous utrophin expression in DMD muscle fibers using a pharmacological intervention. In this case, utrophin-based drug therapy could be administered to patients systemically because utrophin overexpression in tissues other than muscle does not seem to cause detrimental effects [26]. Moreover, utrophin therapy should be effective for all DMD patients, regardless of the specific genetic defect. By contrast, other therapies for DMD, such as aminoglycoside-antibiotic therapy [27] and exon-skipping strategies [12], would have to be tailored to subsets of DMD patients with specific genetic features of the disease.

One of the main differences between utrophin and dystrophin is their contrasting expression patterns. In healthy adult muscle fibers, dystrophin is expressed at high levels along the entire length of the sarcolemma, whereas utrophin expression is confined to the myotendinous [28] and the neuromuscular junction (NMJ) [29]. Therefore, a challenge for a utrophin-based drug therapy is to stimulate expression of utrophin at appropriate levels along the entire sarcolemma of dystrophic muscle fibers, including both synaptic and extrasynaptic compartments. In contrast to the adult expression pattern, utrophin is expressed at high levels at the sarcolemma during development [30] and, hence, precedes expression of dystrophin [31]. This implies that utrophin performs the function of dystrophin in fetal and developing muscle fibers. Drug-based utrophin therapy can thus be regarded as an attempt to re-activate sarcolemmal expression of the fetal form of dystrophin. Such a strategy is not without precedent; indeed, re-activation of fetal hemoglobin is a current therapy for the treatment of various β-hemoglobinopathies [32].

To unlock the therapeutic potential of utrophin as a drug target, a thorough understanding of the mechanisms that regulate its expression is necessary. In the following sections, we highlight several important mechanisms that regulate utrophin at the level of transcription. Subsequently, we summarize the findings of several recent preclinical studies that demonstrate that manipulation of these mechanisms and pathways can alleviate some of the characteristic symptoms associated with the dystrophic phenotype in the mdx mouse.

Mechanisms governing transcription of the gene encoding utrophin

Role of the utrophin-A promoter in regulating synaptic expression of utrophin

Two full-length isoforms of utrophin have been identified: utrophin A is the isoform found in skeletal muscle fibers, whereas utrophin B is confined to the vascular endothelium [33,34]. To prevent muscle damage caused by DMD, expression of utrophin must be increased along the entire length of muscle fibres so that it functions as a
Thus, it is important to understand the elements responsible for the restriction of utrophin-A expression at the NMJ in mature skeletal muscle fibers. Work by several groups has shown that transcription in myonuclei beneath the postsynaptic membrane of the NMJ is an important regulatory event that controls the junctional expression of utrophin. Analysis of the utrophin-A promoter region revealed that it contains an N-box motif [35], which is a crucial element for directing the transcription of genes within the postsynaptic sarcoplasm [36]. Indeed, this element was shown to be important for the synaptic expression of utrophin A [37,38]. This N-box motif is targeted by the ets-related transcription factors known as GA-binding protein α.

Figure 1. Regulatory mechanisms governing utrophin expression. In mature muscle, utrophin is primarily expressed at the postsynaptic sarcoplasm of both fast and slow fibers, although a low level of expression is also detected in the extrasynaptic compartment of slow fibers. At the sarcolemma, utrophin associates with members of the dystrophin-associated protein complex (DAPC), thereby providing a link between the actin cytoskeleton and the extracellular matrix. Within the synaptic region, the nerve-derived-trophic factor heregulin binds to ErbB receptors and activates a cascade that culminates in transcriptional activation of the utrophin promoter A via GABPα and GABPβ, and the N-box motif. The cofactor PGC-1α activates the transcription of GABPα, which in turn promotes utrophin-A transcription. The utrophin-A promoter region also contains sites that bind the zinc-finger transcription factors Sp1 and Sp3. Within the extrasynaptic regions of slow muscle fibers, calcineurin dephosphorylates NFAT, enabling it to enter the myonucleus and activate the utrophin-A promoter. RhoA has been implicated in activating utrophin transcription via unknown mechanisms and in conferring stability to the utrophin-A protein. The 3′-untranslated region (3′-UTR) confers stability to the utrophin transcript and controls its subcellular localization, whereas an internal ribosome entry site (IRES) in the 5′-UTR of the utrophin-A mRNA stimulates utrophin translation during muscle regeneration. Inflammatory signals such as TNF-α are probably involved in regulating the calpain-mediated degradation of utrophin. For simplicity, some members of the DAPC have been omitted.

Abbreviations: α-DG, α-dystroglycan; β-DG, β-dystroglycan; NFAT, nuclear factor of activated T cells; TNF-α, tumor necrosis factor α. ? denotes that the mechanism of action is unknown.
Box 1. Animal models of DMD

The most extensively studied animal model of dystrophin-deficient muscular dystrophy is the mdx mouse. This mouse lacks dystrophin as a result of a spontaneous point mutation, and exhibits a mild pathology compared with the human disease, possibly because of its enhanced muscle-regenerative capacity. Another DMD model is the golden-retriever muscular-dystrophy (GRMD) dog, which exhibits a disease progression that resembles more closely the human disease. The severe pathology of the GRMD dog and its large size make it useful for evaluating the benefits and the toxicity of experimental therapies. In support of the therapeutic potential of utrophin-based treatments for DMD, it has recently been shown that overexpression of utrophin by viral-mediated gene therapy mitigates the dystrophic phenotype of the GRMD dog [82].

(GABPα) and GABPβ [39,40]. To induce utrophin transcription, GABPα and GABPβ are activated by an extracellular signal-related kinase (ERK) pathway via the release of the nerve-derived trophic factor heregulin and its interaction with ErbB tyrosine kinase receptors [39]. In this way, signals from the nerve terminal can direct the expression of utrophin within the postsynaptic sarcoplasm (Figure 1).

In addition to the N-box motif, the promoter region of utrophin A contains sites that are targeted by Sp1 and Sp3 zinc finger-containing transcription factors, which cooperate with GABP to activate transcription [41,42]. There is now evidence that the phosphatase inhibitor okadaic acid regulates activation of the utrophin-A promoter through Sp1 [43]. Adding to the complexity of the events that regulate promoter activation via GABP, the peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1α (PGC-1α) was recently shown to cooperate with GABPα to activate the transcription of utrophin A [44]. PGC-1α is a transcriptional cofactor that is important in driving the formation of slow-twitch muscle fibers [45].

Calcineurin–NFAT signaling regulates utrophin expression in slow muscle fibers

In addition to the mechanisms that regulate the synaptic expression of utrophin, other transcriptional events are important for the expression of utrophin in different muscle types. It has been known for some time that fast fibers of DMD patients are subject to greater damage than their slow counterparts (Glossary) [46]. Moreover, a differential susceptibility to damage between these two muscle types has been observed in the mdx mouse [47]. Some insight into a possible explanation for the reduced damage in the slow, more oxidative muscle type comes from the observation that utrophin is expressed in extrasynaptic regions of these fibers and thus might protect against muscle damage [48]. In support of a role for the slow myogenic program in regulating utrophin levels, it has been shown that stimulation of the slow muscle phenotype by functional overload results in greater expression of utrophin A [34]. Because the Ca2+-dependent and calmodulin-dependent serine/threonine phosphatase calcineurin is an important regulator of the slow myogenic program [49], it was hypothesized, based on these findings, that calcineurin signaling has a major role in regulating utrophin expression. Indeed, calcineurin activation can increase utrophin-A mRNA levels in slow muscle fibers of the mouse, whereas inhibition of calcineurin activity by cyclosporine reduces utrophin-A mRNA levels [34]. Although further work is needed to substantiate the precise molecular events by which calcineurin activation stimulates utrophin expression and to determine whether other transcription factors [e.g. nuclear factor κB (NF-κB)] are involved, it seems that calcineurin exerts at least part of its effects through activation of the transcription factor nuclear factor of activated T cells (NFAT). Indeed, the utrophin-A promoter region contains a NFAT binding site, and overexpression of NFAT induces transcription at the utrophin-A promoter in muscle-cell cultures [34]. Additionally, mutation of the NFAT binding site abolishes this effect [44]. Together, these data suggest that stimulation of the slow myogenic program and increased utrophin levels through activation of calcineurin–NFAT (Figure 1) might be an appropriate therapy to protect against degeneration of dystrophic muscle fibers [34,48].

Compensation for dystrophin deficiency by stimulation of utrophin expression

The mechanisms of transcriptional control discussed earlier provide multiple targets for pharmacological interventions. Knowledge of the utrophin-A promoter has initiated the search for small molecules or peptides that can stimulate transcription of utrophin. Utrophin transcription in muscle fibers of DMD patients might be induced directly through administration of transcription factors and/or cofactors, or indirectly through either administration of growth factors or activation of upstream signaling pathways. Current efforts are mostly focused on targeting the utrophin-A promoter because this isofom is expressed in skeletal muscle. However, therapies based on activating the utrophin-B promoter remain a possibility [50].

Screening for transcriptional activators

A promising approach to identify transcriptional activators of utrophin involves high-throughput screening to identify compounds and small molecules that can activate the utrophin promoters in muscle cells. This approach is currently being pursued in several laboratories. The main advantage of high-throughput screening is that it enables the rapid assessment of thousands of potentially beneficial compounds from chemical libraries [21]. In this approach, candidate molecules are assessed first for their ability to activate the utrophin-A and utrophin-B promoters in cultured cells derived from mdx mice. Then, positive candidates are screened for their ability to increase the endogenous expression of utrophin in cell lines derived from DMD patients and in vivo in mdx mice. These molecules can be assessed further for therapeutic benefit in another animal model of DMD, the golden-retriever muscular-dystrophy (GRMD) dog (Box 1). Optimization of the candidates that induce utrophin expression is then carried out using medicinal-chemistry approaches and rational drug design. This search has reportedly led to the identification of several candidate molecules that activate utrophin transcription. Further assessment of the efficacy
of these compounds might be accomplished by testing their ability to activate reporter activity specifically in muscle fibers from transgenic mice harboring an utrophin-A promoter reporter-gene construct [51].

Heregulin treatment ameliorates the dystrophic phenotype

The knowledge that nerve-derived signaling has a role in regulating utrophin transcription has been exploited recently to devise a small peptide that might be a useful therapeutic drug. As previously mentioned, the nerve-derived growth factor hereregulin can stimulate utrophin transcription through activation of GABPα and GABPβ and their binding to the N-box motif [39,40]. In a recent study, administration of a small peptide region of the hereregulin ectodomain to mdx mice was shown to increase utrophin expression and ameliorate the dystrophic phenotype [52]. Specifically, 4-week-old mdx mice treated for three months showed an approximately threefold increase in utrophin levels compared with untreated mdx mice, with expression of utrophin found throughout the entire length of the sarcolemma. This level of utrophin induction is considered significant because a twofold or threefold increase is sufficient to ameliorate the dystrophic phenotype of the mdx mouse [53]. Given the role of hereregulin in controlling the postsynaptic accumulation of utrophin (see earlier), these findings might at first seem surprising but in fact they suggest the presence of the entire signaling cascade (including ErbB receptors) in extrasynaptic compartments of muscle fibers. Importantly, the observed increase in utrophin expression was accompanied by a reduction in muscle degeneration and inflammation and by a decreased susceptibility to damage induced by lengthening contractions. The improvement in muscle function was deemed to result specifically from the upregulation of utrophin because hereregulin treatment of mice lacking both utrophin and dystrophin failed to reduce the muscle pathology. However, it should be noted that the benefits conferred to muscle fibers were not as dramatic as previous results obtained by somatic gene transfer of utrophin [24]. These shortcomings are clearly outweighed in therapeutic significance by the advantages of a systemic drug therapy using small peptides over gene-based therapies.

Modulation of calcineurin–NFAT signaling

Because calcineurin–NFAT signaling seems to have a role in controlling the expression of utrophin [34], manipulation of this pathway might be used as a therapeutic strategy to achieve increased expression of utrophin in dystrophic muscle. Proof of concept for such an approach came recently from experiments that showed that introduction of a transgene containing an activated form of calcineurin into the muscle of the mdx mouse caused an approximate twofold increase in utrophin levels and significant improvements in several markers of the dystrophic pathology [54]. In particular, dystrophin-deficient muscle fibers that express the activated form of calcineurin showed attenuation in muscle-fiber damage as determined by a reduction in both fiber-size variability and central nucleation. In addition, these mice exhibited decreased inflammation and a restoration of sarcolemmal integrity [54]. Thus, activation of calcineurin or its downstream effector NFAT is a potential therapy for DMD. It will be of interest to see if high-throughput screens for activators of utrophin transcription result in the identification of molecules that act as agonists of calcineurin–NFAT signaling in skeletal muscle. However, the potential for damaging side-effects caused by stimulating calcineurin–NFAT signaling in other tissues must not be overlooked; for example, cardiac hypertrophy is known to be induced by calcineurin [55] and, thus, systematic stimulation of calcineurin signaling via drug therapy might exacerbate the incidence of heart failure in DMD patients. In most DMD and BMD patients, cardiac muscle is affected to varying degrees, with 20% of DMD fatalities resulting from heart failure [56].

As previously mentioned, the only pharmacological therapy currently employed to treat DMD patients is the administration of corticosteroids, including prednisone and its oxazoline derivative deflazacort. Although treatment with corticosteroids is not a long-term solution and is associated with detrimental side-effects (e.g. weight gain, growth suppression and behavioral abnormalities), it can nonetheless extend the length of time a patient remains ambulatory and also improve pulmonary function [57]. Until recently, the mechanisms by which deflazacort mediates its beneficial effects were largely unknown. Interestingly, a recent study has demonstrated that deflazacort stimulates the calcineurin–NFAT pathway in cultured muscle cells [58]. Furthermore, treatment of mdx mice with deflazacort ameliorates the dystrophic phenotype. Importantly, this improvement is concurrent with increased utrophin-A expression. Both the improved phenotype and the increased sarcolemmal utrophin expression are inhibited by treatment of mdx mice with the calcineurin inhibitor cyclosporine. These results suggest that calcineurin–NFAT activation leading to utrophin upregulation is a mechanism by which deflazacort exerts its beneficial effects in DMD patients.

The attenuation of the beneficial deflazacort-induced effects after cyclosporine treatment suggests that the use of this immunosuppressant in gene-based-therapy protocols for DMD might result in confounding, perhaps even detrimental, effects. Based on the above findings, it can be speculated that cyclosporine treatment reduces the protective effects of endogenous utrophin in DMD muscle fibers by inhibiting calcineurin signaling. However, such an effect has not been observed in a recent study [59] possibly because of the insufficient dose of cyclosporine used, which failed to inactivate calcineurin and affect utrophin expression in muscle fibers. It should also be noted that the mechanism of utrophin upregulation via deflazacort-induced calcineurin activation might not be a general mechanism for all corticosteroids because a study showed no evidence for increased calcineurin activation or utrophin stimulation in mdx mice treated with prednisolone [60]. Nonetheless, the results of the study of the effects of deflazacort [58] imply that identification of molecules that act as specific activators of calcineurin–NFAT signaling in skeletal muscle might be useful for treating DMD.
Increasing nitric-oxide levels

As mentioned earlier, one of the signaling proteins that associate with the DAPC is nNOS, an enzyme that catalyzes the production of the potent signaling molecule nitric oxide (NO) (Figure 1) [61]. In dystrophic muscle fibers, nNOS expression at the sarcolemma is considerably reduced and is redistributed to the cytoplasm as a result of disruption of the DAPC [8]. This has led to the hypothesis that nNOS mediates a protective effect against damage in normal muscle and that this effect is compromised in the dystrophic condition. Indeed, over-expression of a nNOS transgene in the muscle of the mdx mouse reduced the extent of the dystrophic pathology [62]. Thus, therapeutics that modulate levels of NO are possible treatments for DMD.

In this context, administration of L-arginine, a substrate for nNOS, has been shown in several recent studies to increase utrophin expression in mdx mice [63–65]. Thus, NO levels seem to regulate the expression of utrophin in dystrophic muscle fibers. Interestingly, this increase in utrophin expression is concurrent with improvements in the dystrophic pathology. Specifically, mdx mice treated with L-arginine showed reduced necrosis [65] and contractile-induced damage [64]. Although the level of utrophin upregulation achieved through L-arginine treatment of mdx mice varies, twofold or threefold increases have been reported [65]. Thus, treatment with L-arginine or other modulators of nNOS activity might be used as potential therapeutics for DMD. To this end, treatment of mdx mice with the NO donor molsidomine (a drug currently employed in the treatment of various cardiac diseases) produced benefits similar to those induced by L-arginine treatment [65].

It is still unknown how L-arginine treatment results in increased utrophin levels in mdx muscle fibers and thus it is important to determine whether this increase happens at a transcriptional level, or by other mechanisms, such as inhibition of calpain-mediated utrophin degradation [66]. Furthermore, it has not been established that the observed improvements following L-arginine treatment are caused specifically by stimulation of utrophin expression. In addition to stimulation of utrophin levels, NO-based treatments have the potential to benefit dystrophic muscle by reducing inflammation [67], normalizing vasodilation [68] and promoting muscle repair [69]. Thus, NO-based therapy is a potentially multi-targeted approach to treat DMD.

Future directions and perspectives

The three approaches discussed in this article can increase endogenous levels of utrophin and ameliorate the pathology associated with the dystrophic phenotype. The challenge now is to build upon these findings and translate them into effective long-term pharmacotherapeutics for DMD patients (Box 2). Administration of the heregulin peptide is a plausible strategy for treating DMD but requires further long-term assessment of its toxicity and tissue specificity. This assessment will be essential in determining the viability of this approach because detrimental heterogeneous effects might be caused by the activation of various ErbB receptor-dependent signaling cascades and downstream target genes, particularly those involved in cancer [70]. There are fewer obstacles for the clinical assessment of a drug that stimulates NO levels because drugs that increase NO levels (e.g. molsidomine) are currently used for other diseases. Attention must be paid to targeting skeletal muscle for such a therapy because NO signaling has important roles in most tissues. As for the therapeutic potential of activating the calcineurin–NFAT pathway, progression of a drug therapy awaits, in this case, the synthesis of new clinically relevant modulators of calcineurin and/or NFAT signaling [71]. Although these potential drug-based therapies might induce significant side-effects, these are clearly outweighed by the dire need for effective therapies to slow and alter the devastating progression of DMD. Accordingly, such potential drawbacks should not preclude continued efforts to search for effective molecules leading to utrophin upregulation in dystrophic skeletal muscle fibers.

While the drug therapies outlined here undergo further assessment and optimization, it is important to continue searching for additional regulatory pathways that modulate utrophin transcription. Given the recently demonstrated role of PGC-1α in regulating utrophin transcription [44], it will be interesting to assess how established regulators of PGC-1α affect utrophin transcription. In this context, the myocyte enhancer factor 2 (MEF2) family of transcription factors are known to stimulate expression of PGC-1α in a positive-feedback regulatory mechanism [72]. Transcription of MEF2 is also known to be regulated by multiple Ca 2+ -dependent signaling networks, including the calcineurin pathway [73]. In addition, MEF2 transcription is suppressed by class II histone deacetylase proteins (HDACs) [74]. Thus, inhibition of HDAC activity might result in increased MEF2 levels and stimulation of PGC-1α expression, ultimately leading to increased utrophin levels. This strategy is of particular interest because HDAC inhibitors are in clinical use as anti-neoplastic agents [75].

Box 2. Outstanding questions

- Because embryonic muscle fibers express high levels of utrophin throughout the entire length of the sarcolemma, what are the mechanisms leading to the repression of utrophin expression in the extrasynaptic compartments of adult fast muscle fibers?
- In addition to NFAT, does calcineurin stimulate transcription of utrophin in skeletal muscle through other transcription factors (e.g. MEF2 and NF-κB)?
- Does calcineurin also contribute to maintaining high levels of utrophin transcript through post-transcriptional mechanisms operating at the level of mRNA stability?
- Is the improved phenotype of mdx mice treated with L-arginine a direct result of increased utrophin levels?
- Is utrophin B as effective as utrophin A in functionally compensating for the lack of dystrophin in dystrophic muscle fibers? If so, how can activation of the utrophin-B promoter be achieved, and what are the mechanisms controlling transcription of utrophin B?
- Will long-term studies of utrophin that stimulate pharmacological treatments in the mouse and dog models of muscular dystrophy prove successful?
- During what stage of the progression of DMD will utrophin-stimulating drug therapies be effective for patients?
set of clinically approved drugs, the PPAR agonists [76], might also be used to modulate utrophin levels. Because PGC-1α is a cofactor for PPAR-γ, and agonists of PPAR-γ are known to induce a switch of fast muscle fibers towards the slow, more oxidative muscle fiber type in mice [77], it will also be important to assess the effect of various PPAR agonists on utrophin expression and the dystrophic-muscle pathology.

In addition to transcriptional control mechanisms, recent work has shown that utrophin is subject to regulation at multiple steps of its synthesis and degradation (Figure 1). For example, at the post-transcriptional level, the 3′-untranslated region (3′-UTR) of the utrophin transcript is important in mediating its stability and subcellular localization [48,78]. At the level of translation initiation, an internal ribosome entry site (IRES) was found within the utrophin-A 5′-UTR and shown to regulate expression of utrophin during muscle regeneration [79]. Moreover, utrophin seems to be controlled by post-translational mechanisms; specifically, the GTPase RhoA stabilizes utrophin protein in muscle-cell cultures [80], and there is also evidence suggesting that utrophin levels are regulated by calpain-mediated proteolysis [66]. Finally, inflammatory signaling seems to have a role in controlling utrophin expression through unknown regulatory mechanisms [66,81]. With the discovery of these multiple levels of additional regulation comes the opportunity for directing pharmacological interventions towards a wealth of novel targets.

Concluding remarks

The ongoing investigation into the various mechanisms that control the expression of utrophin has led to the recent findings that treatment with heregulin, activation of calcineurin–NFAT signaling and increased NO levels can all stimulate utrophin expression and mitigate the dystrophic phenotype of the mdx mouse. These encouraging preclinical results provide the impetus to develop new utrophin-stimulating drugs and to continue the elucidation of regulatory mechanisms that govern utrophin expression, particularly with regard to the effect of calcineurin on utrophin levels. Although the time for using utrophin-based drug therapy to treat DMD patients has not yet arrived, the significant progress made recently has brought us much closer to the implementation of utrophin-stimulating pharmacotherapies. Additional long-term studies are needed to assess the benefits and toxicity of such drugs in both mouse and dog models of DMD (Box 1). It should be noted that a utrophin-based therapy should not be used in isolation but should be combined with other interventions. For example, it has been proposed that augmenting utrophin levels could be combined with blockade of myostatin [52]. Such a combinatorial therapy will hopefully significantly improve the quality and duration of life of people affected by DMD. The lack of effective therapies and drugs for treating this debilitating disease highlights the need for additional investigation into the therapeutic potential of utrophin.

References

8 Brennan, J.E. et al. (1985) Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. Cell 82, 743–752
22 Perkins, K.J. and Davies, K.E. (2002) The role of utrophin in the potential therapy of Duchenne muscular dystrophy. Neuromuscul. Disorders. 12(Suppl. 1), S78–S89
46 Webster, C. et al. (1988) Fast muscle fibres are preferentially affected in Duchenne muscular dystrophy. J. Biol. Chem. 263, 743–751