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# Upregulation of paxillin and focal adhesion signaling follows Dystroglycan Complex deletions and promotes a hypertensive state of differentiation

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ABSTRACT

Anchorage to matrix is mediated for many cells not only by integrin-based focal adhesions but also by a parallel assembly of integral and peripheral membrane proteins known as the Dystroglycan Complex. Deficiencies in either dystrophin (*mdx* mice) or γ-sarcoglycan (γSG$^{-/-}$ mice) components of the Dystroglycan Complex lead to upregulation of numerous focal adhesion proteins, and the phosphoprotein paxillin proves to be among the most prominent. In *mdx* muscle, paxillin-Y31 and Y118 are both hyper-phosphorylated as are key sites in focal adhesion kinase (FAK) and the stretch-stimulatable pro-survival MAPK pathway, whereas γSG$^{-/-}$ muscle exhibits more erratic hyper-phosphorylation. In cultured myotubes, cell tension generated by myosin-II appears required for localization of paxillin to adhesions while vinculin appears more stably integrated. Overexpression of wild-type (WT) paxillin has no obvious effect on focal adhesion density or the physical strength of adhesion, but WT and a Y118F mutant promote contractile sarcomere formation whereas a Y31F mutant shows no effect, implicating Y31 in striation. Self-peeling of cells as well as Atomic Force Microscopy (AFM) probing of cells with or without myosin-II inhibition indicate an increase in cell tension within paxillin-overexpressing cells. However, prednisolone, a first-line glucocorticoid for muscular dystrophies, decreases cell tension without affecting paxillin at adhesions, suggesting a non-linear relationship between paxillin and cell tension. Hypertension that results from upregulation of integrin adhesions is thus a natural and treatable outcome of Dystroglycan Complex down-regulation.



## Introduction

Tissue cells not only attach to but also pull on matrix as part of 'tactile' signaling mechanisms (Discher et al., 2005). Myosins invariably provide the pulling force in establishing a cytoskeletal tension, and cell anchorage generally occurs *via* the well-studied integrin-based focal adhesion system but *also* – in many cell types – *via* the Dystroglycan Complex (DGC). Identified first in myocytes (Campbell, 1995), the DGC is increasingly understood to be used by many cells (Campbell, 1995; Muschler et al., 2002) for anchorage to basal lamina. Integrin ↔ DGC signaling appears bidirectional (Yoshida et al., 1998), and yet the interplay with cell tension and contractility is unknown, as is any impact on cell differentiation.

The DGC linkage between the cytoskeleton and the extracellular matrix (ECM) is often perturbed or disrupted in muscular dystrophies (MD). Myoblasts are relatively unaffected because the DGC is expressed only in post-fusion, non-dividing myotubes, but tension-induced damage to the mature muscle membrane ultimately causes muscle weakness, massive degeneration, and premature death (Campbell, 1995; Straub and Campbell, 1997; Lim and Campbell, 1998; Cohn and Campbell, 2000). Importantly, in both dystrophin-deficient patients (Duchenne Muscular Dystrophy) as well as in dystrophin-deficient *mdx* mice, the contractile myotubes partially compensate for the lack of an intact DGC by up-regulating integrins, particularly α7β1 (Fig. 1A, right sketch) (Hodges et al., 1997). An intermediate level of compensation occurs with deficiency of the DGC component γ-sarcoglycan, leading to what also appears to be a more apoptotic phenotype (Griffin et al., 2005). Intentional overexpression of α7β1 has proven protective (Yoshida et al., 1998; Allikian et al., 2004; Burkin et al., 2005), but whether this is strictly from stabilizing transmembrane force transmission or also from the recruitment of additional cytosolic proteins to the integrin complex has not yet been addressed.

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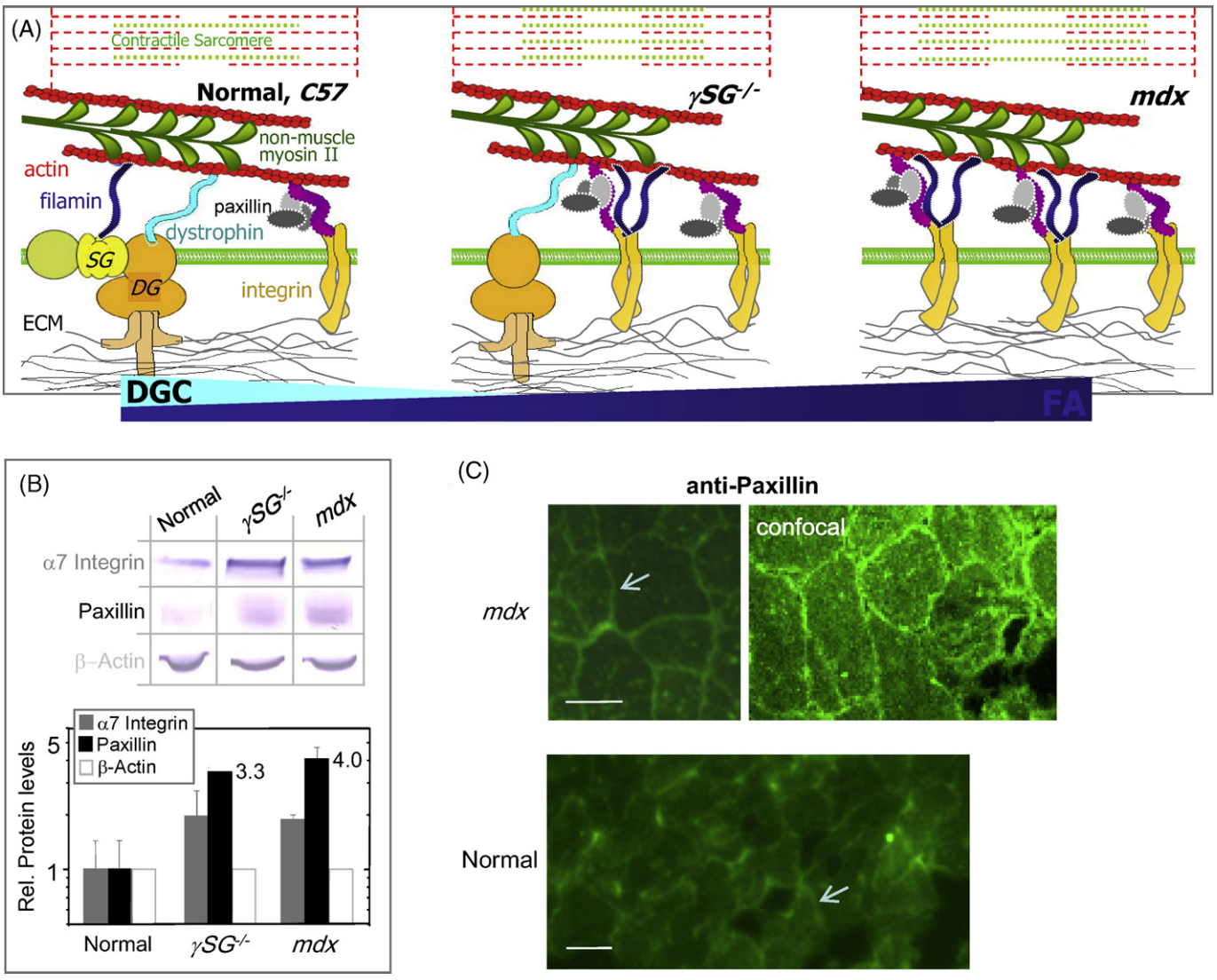


**Fig. 1.** Upregulated adhesive complex components in muscular dystrophies. (A) Sarcolemma scaffolding in normal (C57), γSG$^{-/-}$, and *mdx* muscle cells illustrates the shift in cell attachment mediated by Dystroglycan Complex (DGC) to increasingly integrin-based focal adhesions (FAs). (B) Western blot analysis of C57, *mdx* and γSG$^{-/-}$ muscle lysates shows a 2-fold upregulation of α7-integrins in dystrophic muscle together with 4–5-fold increases in paxillin ($n = 3$). β-Actin is used to normalize, and in so doing the integrin result appears consistent with past reports (Hodges et al., 1997). (C) Muscle sections immunostained with monoclonal anti-paxillin plus secondary and imaged under identical conditions by conventional fluorescence microscopy and confocal (*mdx*). Secondary alone shows no labeling. Paxillin intensity in normal muscle appears dim relative to *mdx*, and only the latter showed sufficient intensity to be imaged by laser scanning confocal, suggesting paxillin localization to sarcolemma (arrows) as well as cytosol. Scale bars ~20 μm.

Talin, α-actinin, and perhaps filamin contribute scaffolding roles in integrin-based focal adhesions (FAs), whereas other components such as paxillin, vinculin, and FAK diffuse in and out as part of a phospho-tyrosine based signaling nexus (Panetti, 2002; Shemesh et al., 2005; Zaidel-Bar et al., 2007; Pasapera et al., 2010). Essential for embryonic development (Furuta et al., 1995; Xu et al., 1998; Hagel et al., 2002; Charlesworth et al., 2006), FA-derived signals promote assembly of cytoskeletal tension structures such as stress fibers and also propagate cell survival signals into the MAPK pathway (Turner, 2000; Hagel et al., 2002; Brown and Turner, 2004) with activation of ERK (Fluck et al., 1999; Turner, 2000; Most et al., 2003; Schaeffer et al., 2003; Lunn and Rozengurt, 2004; Melendez et al., 2004; Mizukami et al., 2004; Subauste et al., 2004; Lin et al., 2005; Palfi et al., 2005; Vittal et al., 2005; Das et al., 2006; Peng et al., 2006; Wei et al., 2006) – which is already known to be enhanced in stretched *mdx* muscle (Kumar et al., 2004) and in γSG$^{-/-}$ muscle (Griffin et al., 2005). In maturing myotubes, FAs are the nucleation sites for myofibrillogenesis (McKenna et al., 1986; Sanger et al., 2002) during which extensive cytoskeletal remodeling ultimately replaces non-muscle myosin-II (NMM-II) mini-filaments with the contractile striations of skeletal muscle myosin-II (Fig. 1A). Given the upregulation of integrins in muscular dystrophies, as well as the known enrichment of filamin at the sarcolemma of both *mdx* and γSG$^{-/-}$ mice (Thompson et al., 2000), we hypothesized that additional FA components would also be modulated and would influence downstream outputs ranging from cell tension and myofibrillogenesis to viability. Transcript profiles of *mdx* versus normal muscle (Bakay et al., 2002) indeed hint at increases in paxillin (+15%; see Table S1A) as well as other components, such as vinculin (+35%) and γ-actin, and the latter has recently been shown to be elevated at the protein level (Hanft et al., 2006). Signaling and phenotype depend on protein levels, post-translational modifications, and collective interactions with feedback loops in and between signaling networks. Here we demonstrate – as part of compensatory mechanisms within mouse dystrophic muscle – a major upregulation of paxillin and adhesive signaling that promotes general