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Dystrophin as a focal adhesion protein

Collocalization with talin and the M_r 48000 sarcolemmal protein in cultured *Xenopus* muscle

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Monoclonal antibodies against dystrophin and the postsynaptic 58 kDa protein from *Torpedo* electric organ were used to localize homologs of these proteins in cultured skeletal muscle (*Xenopus laevis*). The *Xenopus* homolog is an M_r 48000 protein and, like dystrophin, is a sarcolemmal protein. Both proteins localized precisely to talin-positive sites, hence with each other, on the substrate-apposed sarcolemma. Therefore, the first sites of appearance of dystrophin on cultured muscle cells are focal adhesions, i.e. specific sites of cytoskeleton/extracellular matrix interaction. These data also add to evidence that dystrophin and the 58 kDa act together.

Sarcolemma; Duchenne muscular dystrophy; Cytoskeleton; Membrane protein skeletal network; *Xenopus laevis*

1. INTRODUCTION

The causative biochemical defect in Duchenne and Becker muscular dystrophy lies in dystrophin, a cytoplasmic sarcolemmal protein of normal skeletal, cardiac and smooth muscle (reviewed in [1,2]). Dystrophin is a member of the spectrin/α-actinin family of actin binding proteins with a predicted monomer length of 150 nm [3]. Current hypotheses suppose that dystrophin prevents the membrane and internal damage seen in Duchenne muscle cells [4] through mechanical stabilization of the plasma membrane (in analogy to erythrocyte spectrin) or by regulating calcium-permeable membrane channels [5].

Very little is known about the membrane sites at which dystrophin accumulates. However, dystrophin surrounds and interdigitates with acetylcholine receptor (AChR) domains at AChR clusters in cultured skeletal muscle from *Xenopus laevis* [6]. This is also characteristic of several proteins of focal adhesions (attachments of cultured cells to their substrata) [7]. Here we have more directly explored the possible relationship between dystrophin and focal adhesions in cultured *Xenopus* muscle by localizing dystrophin relative to two other proteins. One is talin, a protein of focal adhesions in many cultured cells [8] and of the myotendinous junction in skeletal muscle [9,10]. The second is the *Xenopus* muscle homolog of the 58 kDa postsynaptic protein of electric tissue [11]. This protein, designated 48 kDa [12] in this paper, is also a component of the myotendinous junction (as is dystrophin [13]) and talin-positive sites in cultured *Xenopus* muscle [12]. The results confirm that the earliest sites of dystrophin accumulation in cultured muscle are focal adhesions and add to growing indirect evidence [14] that the electric tissue 58 kDa protein and its muscle homologs interact with dystrophin.

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2. MATERIALS AND METHODS

2.1. *Cultures*

Myotomal (tail axial) muscle from stage 20–22 *Xenopus laevis* embryos were cultured at 10°C as previously described [6].

2.2. *Antibodies*

Two rabbit antisera against chicken gizzard talin were used interchangeably: serum A2 [15], shown by Western blot analysis to recognize rat [16] and *Xenopus* muscle talin [12] specifically, and serum 1636 [10]. Mouse monoclonal antibody 1958 against *Torpedo* dystrophin [17] recognizes muscle dystrophin from several species [6]. The reactivity of monoclonal antibody 1351, raised against the *Torpedo* 58 kDa protein [11], with the *Xenopus* 48 kDa homolog has been described [12]. Fluorescein and rhodamine-conjugated anti-mouse and anti-rabbit IgG second antibodies (Jackson ImmunoResearch) were adsorbed against rabbit and mouse IgG, respectively, before use.

2.3. *Immunofluorescence*

Cultures were fixed in cold 95% ethanol, then double-labeled for immunofluorescence by standard methods [18] using 0.8% BSA/1% fish gelatin (Sigma Chemical Co., cat. no. G-7765) in PBS for incubations and washes.

3. RESULTS

Rabbit anti-talin antibodies labeled both skeletal muscle and fibroblastic cells in *Xenopus* muscle

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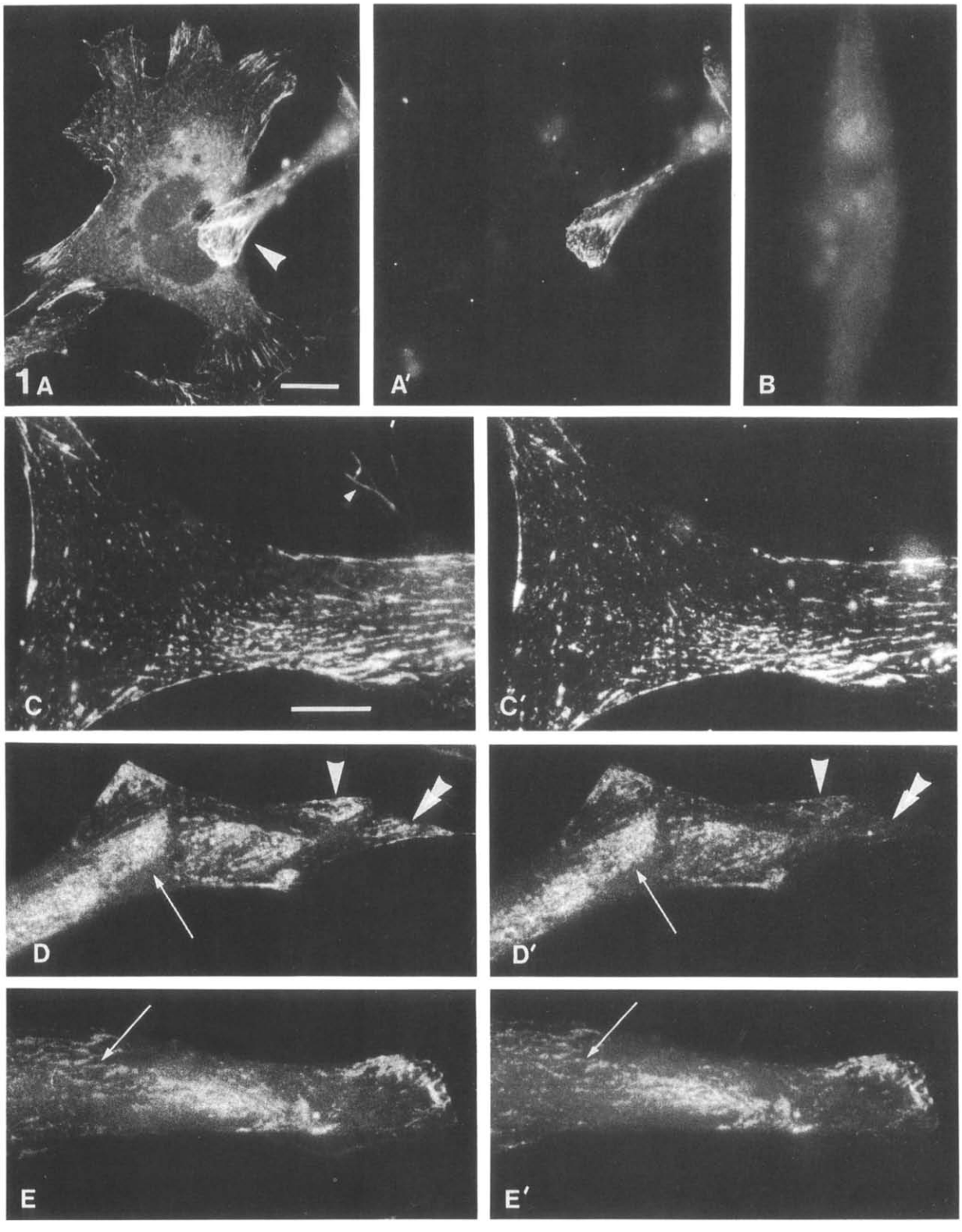

Fig. 1. Talin and dystrophin in double-labeled *Xenopus* muscle cultures. Unprimed letters in each pair of images: anti-talin antibodies. Primed letters: anti-dystrophin mab 1958. Cultures were 2 days old (B,C,C′) or 20 days old (A,A′,D–E′). (A,A′) A muscle cell (arrowhead) growing under a fibroblast which was labeled only by the anti-talin antibodies. (B) Cultures treated with mab 1958 followed by anti-rabbit IgG second antibody were not labeled (a muscle cell is shown). Arrowhead in C,C′: a talin-positive, dystrophin-negative filopodia. Single arrowheads in D,D′: a talin-positive zone that was only weakly positive for dystrophin. Double arrowheads: a talin-positive zone negative for dystrophin. Arrows in E,E′: a field of fine streaks in a 20-day-old culture. Bar in A (10 μm) applies to A,A′,B. Bar in C (10 μm) applies to C–E′.

cultures (Fig. 1A), while the anti-dystrophin mab labeled only muscle cells (Fig. 1A'). When cultures were labeled with the mouse anti-dystrophin mab alone followed by anti-rabbit IgG second antibody, no labeling of muscle cells was detected (Fig. 1B). These results show that cross-reactions between the primary and secondary antibodies were negligible.

Talin was detected on the ventral (substrate-attached) surfaces of the muscle cells in highly variable patterns that were quite distinct from the discrete spots characteristic of fibroblasts (Fig. 1A; cf. [15,19]). After 2 days of culture, the anti-talin staining ranged from weak and diffusely or poorly organized (not shown) to moderately strong and organized in distinct, usually fine, streaks (Fig. 1C). In older cultures, the staining was stronger and more often organized in definite patches (Fig. 1D,E), although fields of fine streaks were still present (Fig. 1E,E'). In both young and old cultures, dystrophin was detected only at talin-positive sites (Fig. 1A,A',C',D',E'). Close comparison of the images showed that the dystrophin distribution was virtually identical to the talin distribution.

The correspondence of the talin and dystrophin distributions held for most of the ventral surface in almost all muscle cells. However, small areas could be found that were positive for talin but negative for dystrophin (single arrowheads in Fig. 1D,D') or only weakly positive for dystrophin (double arrowheads in fig. 1D,D'). Features that would allow us to predict such sites on the ventral surfaces were not identified. However, fine, talin-positive filopodia which developed from occasional muscle cells were invariably negative for dystrophin (Fig. 1C,C').

We have shown previously, at moderate resolution, that talin and the 48 kDa protein are similarly distributed in cultured *Xenopus* muscle [12]. Here we found that, with two exceptions, the 48 kDa protein occurs at talin-positive sites in muscle cells to a very high level of detail (Fig. 2A,A'). The exceptions were the filopodia, which did not stain for the 48 kDa protein (not shown), and small, well-circumscribed areas that were more uniformly stained by mab 1351 than by anti-talin antibodies (Fig. 2A'). Such areas were not found with anti-dystrophin staining. They were presumably AChR clusters, since the mouse and *Xenopus* muscle homologs of the 58 kDa protein are known to give strong labeling of AChR domains within clusters [11]. Dystrophin and talin, however, do not overlap AChR domains [6,7].

4. DISCUSSION

Dystrophin has previously been shown to occur on patches of unknown nature on cultured human muscle [20]. The present results indicate that dystrophin-positive sites in the *Xenopus* cultures are focal adhesions, i.e. sites of closest contact between the ventral surface and the substrate at which force is transmitted from the cytoskeleton to the extracellular matrix [8]. This identification is possible because talin is a key component of focal adhesions in many cultured cells [8] and actin filaments (stress fibers) have been shown to project to talin-positive sites in cultured *Xenopus* muscle [12] as they do at focal adhesions in fibroblasts. The function of dystrophin at these sites is unknown. It is presumably not necessary for force transmission, since it is absent at fibroblast focal adhesions (Fig. 1A,A'; fibroblasts do not synthesize dystrophin [21]), and fibroblast focal adhesions do not contain spectrin [8], the known protein whose structure most resembles the predicted structure of dystrophin. Dystrophin, a probable actin binding protein [22], could possibly be non-functionally bound to the stress fibers where they insert into focal adhesions. However, the absence of dystrophin from the remaining length of the stress fibers and its absence from occasional talin-positive sites (Fig. 1D,D') suggest it is subject to specific regulatory influences. This implies that dystrophin acts at focal adhesions as an integral part of a complex structure.

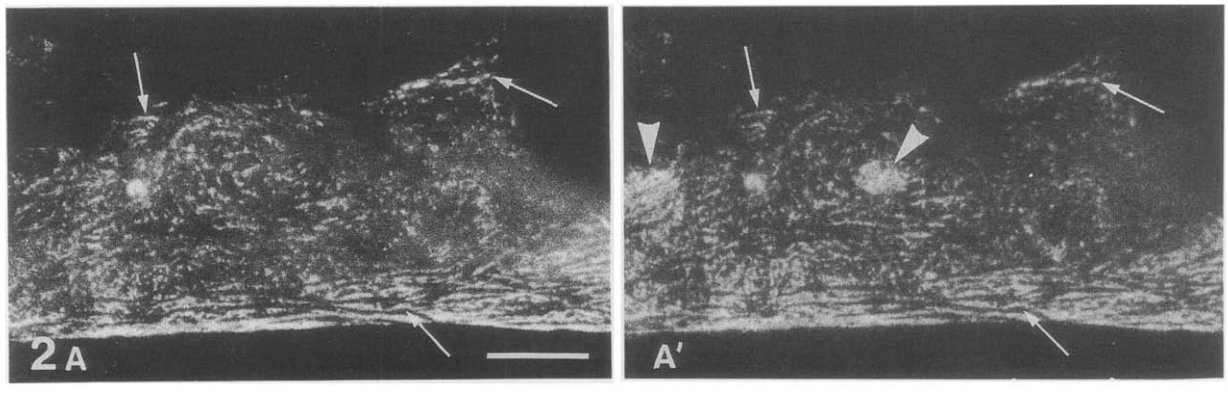

Fig. 2. Talin and the 48 kDa protein in double-labeled cultures (2-day). (A) Talin. (A') 48 kDa protein (mab 1351). Arrows indicate examples of identical details in the two images. Arrowheads in A': local areas rich in 48 kDa protein but not in talin which are probably AChR clusters. Bar = 10 μm.

Since both dystrophin and the 48 kDa protein collocalize precisely with talin over most of the ventral surface of cultured muscle cells, they collocalize with each other. In addition, dystrophin and the 58 kDa/48 kDa protein are similarly distributed on the electroplaque postsynaptic membrane [6,11] and over the entire sarcolemma of normal skeletal, cardiac, and smooth muscles ([11], N.R.K. and R.S., unpublished results). The mouse homolog of the 58 kDa protein is also present in muscle of the mdx mouse (a dystrophin-minus mutant [23]), but is not incorporated onto the sarcolemma [14]. Finally, the 58 kDa protein copurifies with *Torpedo* dystrophin [14]. These observations strongly suggest a direct association between dystrophin and the 58 kDa/48 kDa protein. Interestingly, the 58 kDa/48 kDa protein is also a component of AChR domains in AChR clusters, while dystrophin is not. This would leave the proposed dystrophin binding site on the 48 kDa protein unoccupied. However, AChR domains also contain an unusual form of β-spectrin [24] which is apparently not accompanied by an α-subunit and may therefore exist as a homodimer [24]. Dystrophin is also expected to exist as a homodimer [1]. All the presently available data are therefore compatible with association of the muscle homologs of the 58 kDa protein with this β-spectrin and with dystrophin, presumably at homologous sites on the two proteins.

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