Immunogold localization of adhalin, α-dystroglycan and laminin in normal and dystrophic skeletal muscle.

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Adhalin and α-dystroglycan are two components of a complex of proteins that, in conjunction with dystrophin, provide a link between the subsarcolemmal cytoskeleton and the basal lamina, (part of the extracellular matrix of skeletal muscle) [1, 2]. In the absence of dystrophin, in Duchenne muscular dystrophy (DMD) and the *mdx* mouse, levels of adhalin, α-dystroglycan and other components of the complex, are severely reduced. Adhalin deficiency is also associated with limb girdle muscular dystrophy type 2C and is linked with an unidentified gene on chromosome 13 while limb girdle dystrophy type 2D is associated with mutations in the adhalin gene on chromosome 17. Moreover a proportion of cases of classical congential muscular dystrophy have recently been associated with a deficit in the expression of laminin [3] to which α-dystroglycan normally binds [4].

Models of the organisation of the dystrophin-glycoprotein complex are largely based on biochemical data, and hitherto there has been no ultrastructural information on the localization of adhalin and α-dystroglycan. Immunogold techniques have been used to localise dystrophin [5-7] and, more recently, β-dystroglycan [8]. We here report the use of single and double immunogold labelling to examine the subcellular positioning of adhalin, α-dystroglycan and laminin in control and dystrophic human muscle.

The following monoclonal antibodies were used: (1) Anti-adhalin antibody $IVD3_1$ [9]. Isotype; mouse IgG_1. (2) Anti-α-dystroglycan antibody IIH6 [4]. Isotype; mouse IgM. For double labelling experiments, the following polyclonal antibodies were also used: (1) Affinity purified rabbit polyclonal antibody (P1461) raised against the last 17 C-terminus amino acids of dystrophin [10]. (2) Affinitiy purified rabbit polyclonal antibody to laminin (Sigma, code L-9393). The muscle samples examined (6 DMD cases; 10 control cases) were obtained by open biopsy from the vastus lateralis muscle from patients at the Regional Neurosciences Centre, Newcastle General Hospital. Our methods for carrying out immunolabelling are detailed in several publications [5, 6, 8]. To carry out double labelling, the sections were first labelled by the usual procedure for single labelling with one of the antibodies (monoclonal or polyclonal) and the appropriate gold-conjugate and, after washing, were then labelled with the second antibody (raised in a different species to the first antibody) and a gold-conjugate of a different size to that used for the first antibody. In order to control for possible cross-reactivity or steric hindrance, the sequence of labelling for the two antibodies was reversed.

Using the adhalin antibody, $IVD3_1$, immunogold labelling was seen at the edges of the fibres, mostly close to the plasma membrane and almost entirely on the outer face of it (Fig. 1). This is consistent with biochemical data suggesting that $IVD3_1$ recognises an extracellular epitope (unpublished data). Using the α-dystroglycan antibody, IIH6, immunogold labelling was found on the outer side of the plasma membrane, usually at a site where material that was denser than the background could be seen projecting from the outer face (Fig. 2).

When double labelling with laminin was carried out, the 10nm gold probe for laminin was mostly seen on the proximal side of the basal lamina facing the labelling sites for α-dystroglycan (Fig. 2). When double labelling of α-dystroglycan and dystrophin was carried out (not shown) there was a clear topographical separation of the two labelling sites with the dystrophin probe either on the membrane or immediately internal to it and the α-dystroglycan probe. In DMD muscle labelling of adhalin and α-dystroglycan was found in the same position as in control muscle but at a far lower density, whereas the intensity of laminin labelling showed no difference.

Based on a great deal of biochemical evidence, several schematic models have been proposed for the structure of the dystrophin-

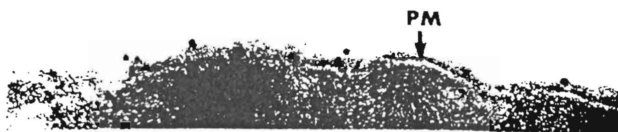

Fig. 1. Immunogold labelling of adhalin in skeletal muscle. Cryosection. PM = plasma membrane. Scale bar = 100nm.

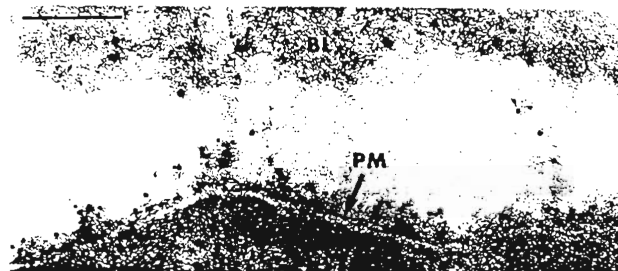

Fig. 2. Double labelling of α-dystroglycan (5nm gold) and laminin (10nm gold) PM = plasma membrane. BL = basal lamina. Bar = 100nm

glycoprotein complex at the plasma membrane [11-13]. There are a number of specific differences between these models, but certain general features are common to each one. For example, in every model, α-dystroglycan is considered to be positioned on the extracellular side of the plasma membrane where it binds to laminin located in the basal lamina. Also, adhalin, while classified as an integral membrane protein, is considered to have a large extracellular component. The immunogold data presented here provide information that is not obtainable by other means and morphological substantiation for models based on biochemical approaches.

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1. Campbell, K. P. & Kahl, S. D. (1989) Nature **338**, 259-262
2. Yoshida, M. & Ozawa, E. (1990) J. Biochem. **108**, 748-752
3. Campbell, K. P. (1995) Cell **80**, 675-679
4. Ervasti, J. M. & Campbell, K.P. (1993) J. Cell Biol. **122**, 809-823
5. Cullen, M., Walsh, J., Nicholson, L.V.B. & Harris, J. B. (1991) Proc. R. Soc. Lond. B. **240**, 197-210
6. Cullen, M. J., Zubrzycka-Gaarn, E. E., Nicholson, L.V.B., Harris, J.B., Ray, P. N. & Worton, R. G. (1991) Neuromusc. Disord. **1**, 113-119
7. Watkins, S. C., Hoffman, E. P., Slayter, H. S. & Kunkel, L. M. (1988) Nature 333, 863-866
8. Cullen, M. J., Walsh, J. & Nicholson, L.V.B. (1994) Acta Neuropathol. **87**, 349-354
9. Jorgensen, A. O., Arnold, W., Shen, AC-Y., Yuan, S., Gaver, M. & Campbell, K.P. (1990) J. Cell Biol. **110**, 1173-1185
10. Zubrzycka-Gaarn, E. E., Bulman, D. E., Karpati, G., Burghes, A.H.M., Belfall, B., Klamut, H. J., Talbot, J., Hodges, R. S., Ray, P. N. & Worton, R. G. (1988) Nature **333**, 466-469
11. Ahn, A. H. & Kunkel, L.M. (1993) Nature Genet. **3**, 283-291
12. Matsumura, K. & Campbell, K.P. (1994) Muscle Nerve **17**, 12-15
13. Suzuki, A., Yoshida, M., Hayashi, K., Mizuno, Y., Hagiwara, Y. & Ozawa, E. (1994) Europ. J. Biochem. **220**, 283-292