

- 3 van Hooff, J. A. R. A. M., in: *Handbook of Methods in Nonverbal Behavior Research*, p. 362. Eds K. R. Scherer and P. Ekman. Cambridge, Cambridge University Press 1982.
- 4 van Engeland, H., Bodnar, F. A., and Bolhuis, G., *J. Child Psychol. Psychiat.* 26 (1985) 879.
- 5 Niesink, R. J. M., and van Ree, J. M., *Science* 221 (1983) 960.
- 6 Spruyt, B. M., *Neurobiol. Aging* 13 (1991) 153.
- 7 Sandman, C. A., Walker, B. B., and Lawton, C. A., *Peptides* 1 (1980) 109.
- 8 De Wied, D., and Jolles, J., *Physiol. Rev.* 62 (1982) 976.
- 9 Pigache, R. M., and Rigter, H., *Front. Horm. Res.* 8 (1981) 193.
- 10 Courchesne, E., Lincoln, A. L., Kilman, B. A., and Galambos, R., *J. Aut. Dev. Dis.*, 15 (1985) 55.
- 11 van Engeland, H., Roelofs, J. W., Verbaten, M. N., and Slangen, J. L., *Psychiat. Res.* 38 (1991) 27.
- 12 Verbaten, M. N., Roelofs, J. W., van Engeland, H., Kenemans, J. L., and Slangen, J. L., *J. Aut. Dev. Dis.* 21 (1991) 449.
- 13 Aman, M. G., Singh, N. N., Stewart, A. W., and Field, C. J., *Am. J. Mental Defic.* 89 (1985) 485 and 89 (1985) 492.
- 14 *Psychopharmacology Bulletin*, 21 (1985).

0014-4754/92/040391-04\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1992

Fusion between a myogenic cell in the satellite cell position and undamaged adult myofibre segments

T. A. Robertson, J. M. Papadimitriou and M. D. Grounds

Department of Pathology, University of Western Australia, Nedlands (6009 Australia)

Received 13 August 1991; accepted 17 October 1991

Abstract. In this report we demonstrate for the first time that differentiating myogenic cells, geographically located between the plasmalemma and external lamina of myofibres in the satellite cell position³, can fuse directly with the plasmalemma of undamaged segments of mature myofibres.

Key words. Fusion; electron microscopy; myogenesis; skeletal muscle; in vivo.

Materials and methods

The mid region of the tibialis anterior of a mature (8-week-old) female SJL/J mouse was injured transversely by superficial application of Karnovsky's fixative¹. Four days after injury mice under halothane anaesthesia were perfused with 2.5% glutaraldehyde (in 0.05 M cacodylate buffer pH 7.4) via the left ventricle and muscle samples were removed and placed in fresh fixative for 24 h. They were then postfixed in 1% OsO₄, dehydrated in ethanol and embedded (after critical orientation) in araldite. The blocks were then sectioned with an LKB ultramicrotome and 50-nm sections were mounted on thin bar 200-mesh copper grids, stained in a lead citrate solution and examined in a Philips 410LS transmission electron microscope at an accelerating voltage of 80 kV.

Results

In regenerating skeletal muscle of adult mice examined 4 days after chemical injury¹ numerous myogenic cells including activated satellite cells, myoblasts and myotubes were observed in the space between the plasmalemma and the external lamina of injured myofibres at a substantial distance (> 0.5 mm) from the injury site. Cytoplasmic continuity which is indicative of fusion was observed between a myogenic cell and a closely opposed normal segment of a myofibre (fig. 1). Serial sectioning demonstrated multiple cytoplasmic confluence along the closely apposed membranes of these two cells (figs 2a and 2b). The myogenic cell beneath the external lamina contained both thick (myosin) and thin (actin) filaments with evidence of sarcomeric organisation (fig. 3). This differentiating cell could have been a myoblast, or due to its

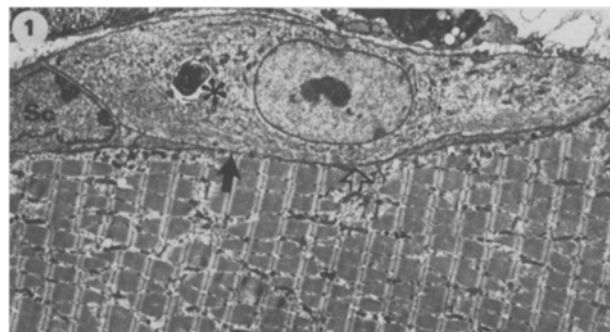


Figure 1. A myogenic cell located between the external lamina and plasmalemma of a myofibre (i.e. in the satellite cell position). Several areas of cytoplasmic confluence (see figs 2a,b,c) between these two apposed cells are marked (open and closed arrows) and a myelinoid body (asterisk) is present in the cytoplasm of this cell. An activated satellite cell (Sc) is also noted beneath the external lamina and plasmalemma of the myofibre. $\times 2100$.



Figure 2a. High magnification of an area of cytoplasmic continuity between the apposing cells (shown in fig. 1 by the closed arrow). After extensive tilting and rotating in a eucentric goniometer, cytoplasmic confluence indicative of fusion can be observed between the two cells at several sites (arrows). $\times 18,840$.

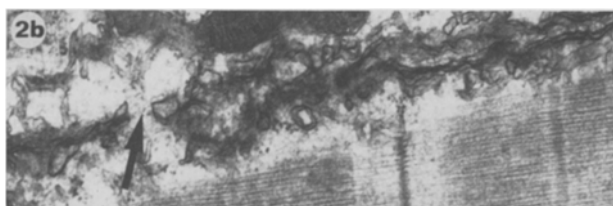


Figure 2b. A serial section (approximately 0.5 μm deeper) of the same area as fig. 2a. Only one site of cytoplasmic confluence can now be seen between the two cells (arrow). Extensive interdigitation of membranes of the two cells is apparent. $\times 25,200$.

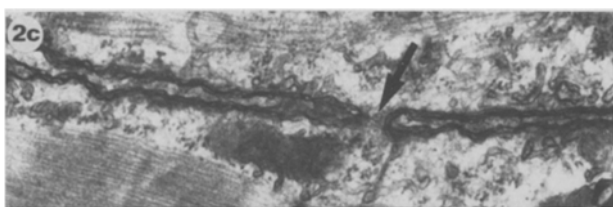


Figure 2c. Another possible fusion site (at the location shown by the open arrow in fig. 1) where cytoplasmic confluence is apparent and the apposing membranes are distinctive (arrow). $\times 25,200$.

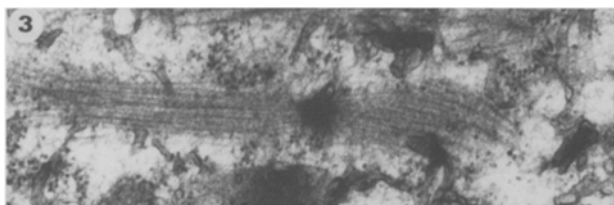


Figure 3. High magnification of the cytoplasm of the myogenic cell located in the satellite cell position, showing thick and thin filaments and evidence of Z-band material. $\times 30,120$.

large size, a myotube although only one nucleus was found. Ultrastructurally there was no evidence of damage to the myofibre such as myofilament disorganisation or mitochondrial abnormalities (fig. 1) at this distance from the injury site.

Discussion

Skeletal muscle regenerates by fusion of mononuclear muscle precursors (myoblasts) to form multinucleated muscle cells (myotubes) and by fusion of these cells with damaged myofibres. A comprehensive study in our laboratory¹ has demonstrated fusion between myogenic cells in vivo in regenerating adult murine skeletal muscle occurred predominately in the injury zone near, or at, the

resealed ends² of damaged myofibres. However, direct fusion with myofibres across an intact external lamina was never seen.

The demonstration of fusion of a myogenic cell in the satellite cell³ position to an apparently undamaged segment of a myofibre is a highly significant finding, as contact of satellite cells with the surface of myofibres has been reported to suppress proliferation and fusion with the myofibre⁴. When skeletal muscle is injured the signal for satellite cell activation must be transmitted a considerable distance along the myofibre to influence satellite cells in that region to proliferate and fuse beneath the external laminal sheath of the myofibre. As demonstrated, these cells can then fuse directly with the plasmalemmal membrane of the myofibre and would contribute significantly to myonuclear numbers and myofilament production. In addition, fusion of myogenic cells with both structurally normal segments of an injured myofibre and with myogenic cells at the site of myofibre damage would result in sarcoplasmic bridges between uninjured segments. Eventually such bridges will enlarge and restore structural continuity to the myofibre affected by segmental necrosis.

The demonstration of fusion between differentiating myogenic cells and apparently undamaged segments of myofibres is of potentially great significance to myoblast transfer therapy, proposed as a treatment for Duchenne Muscular Dystrophy⁵. Injected (normal) donor myoblasts probably fuse with (dystrophic) host myoblasts and myofibres at the site of focal injury and regeneration. We hypothesise that the phenomenon described in this study might also occur along the length of dystrophic myofibres, if donor myoblasts can, in fact, traverse an intact external lamina.

Acknowledgements. We would like to thank Professor John McGeachie for his enthusiastic interest in this work. Support from the National Health and Medical Research Council of Australia (MDG) is gratefully acknowledged.

- 1 Robertson, T. A., Grounds, M. D., Mitchell, C. A., and Papadimitriou, J. M., *J. Struct. Biol.* **105** (1990) 170.
- 2 Papadimitriou, J. M., Robertson, T. A., Mitchell, C. A., and Grounds, M. D., *J. Struct. Biol.* **103** (1990) 124.
- 3 Mauro, A., *J. Biophys. Biochem. Cytol.* **9** (1961) 493.
- 4 Bischoff, R., *Development* **109** (1990) 943.
- 5 Partridge, T. A., *Muscle Nerve* **14** (1991) 197.