Fusion of Myogenic Cells

In Vivo

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After injury to skeletal muscle, effective regeneration is said to depend upon the direct fusion of mononuclear myogenic cells (myoblasts) with the damaged muscle fibres, or fusion between myogenic cells to form multinucleated young muscle cells (myotubes) which subsequently fuse with myofibres (2). The majority of studies on myogenic fusion have been carried out on embryonic tissue culture preparations which do not adequately mimic the vascular responses and the complex three-dimensional cellular interactions between various cell types that occur in vivo. There is also increasing evidence to suggest that the behaviour of myogenic cells derived from embryonic muscle may not be equivalent to that of myogenic precursor cells derived from mature muscle (3). In addition the critical fusion events between myogenic cells and damaged, mature myofibres in regenerating injured muscle cannot be studied because mature fibres cannot be sustained for long in tissue culture. Finally, apart from its relevance in normal muscle regeneration the appreciation of fusion in myogenic cells in vivo has important implications to the current interest in myoblast transfer therapy as a potential treatment for Duchenne Muscular dystrophy (DMD) (4).

In this study electron microscopy was used to investigate myogenic fusion in vivo in adult murine SL/J skeletal muscle regenerating after three different types of injury to the tibialis anterior (TA) muscle, including superficial focal injuries inflicted either by local application of aldehyde, or a cold probe, or the more severe trauma of a crush lesion. All of these have been described in detail in a previous paper (5).
In this report the following terminology for the various myogenic cell types will be adopted: The term "presumptive myoblast" refers to a suspected muscle precursor cell which lacks evidence of cytoplasmic filamentous muscle proteins. A "myoblast" is a muscle precursor cell confirmed by the presence of thick and thin filaments in the cytoplasm. "Mononuclear muscle precursor cell" is a general term which includes satellite cells, presumptive myoblasts and myoblasts. "Myogenic cells" includes both mononuclear muscle precursor cells, multinucleated myotubes and myofibres.

In any ultrastructural identification of small fusion sites, a major problem is difficulty in resolving plasma membranes that are not perpendicular to the plane of section. Although apparent membrane discontinuities in the region of close apposition between myogenic cells may represent actual areas of cytoplasmic continuity, often such appearances merely represent areas where membranes are oblique to the plane of section, and therefore appear as amorphous fuzzy material. Indeed, many of the published electron micrographs, supposedly demonstrating membranes of fusing myogenic cells, fall into this category. A goniometer stage with a rotational holder is essential for adequate manipulation of the specimen to reduce the possibility of misinterpretation. The minimum criteria that should be met for identification of fusion sites are the presence of clearly resolved membrane continuity at either edge of proposed fusion sites (described by Bischoff as "U" shaped membranes) and the complete absence of amorphous material in the area of cytoplasmic confluence (1).

We have carefully documented instances of fusion of myoblast to myoblast, myoblast to myotube and myotube to myotube in a previous paper (5). In summary fusion of these myogenic cells probably begins with intimate apposition of the plasma membranes of the cells, while the appearance of numerous vesicles near the site of cytoplasmic confluence (Fig. 1), often associated with elements of the Golgi apparatus (Fig. 2) suggests that these also play a role. The foci of confluence are often multiple between the two apposed myogenic cells, while persistence near the fusion site of cell membranes, sometimes with remnants of intermediate junctions between them, (Fig. 3) is a frequent feature.

Sarcoplasmic extensions or buds containing variable numbers of myonuclei were often seen at the ends of resealed myofibres as illustrated in Figure 4. An external lamina was absent at the resealed site while the plasmalemmal membrane of myoblasts and myotubes was consistently closely apposed to the sarcolemma of these protrusions. In instances where the bud had acquired a slender finger-like form (Fig. 5 & 7), myotubes were often closely apposed to it (Fig. 6), and often various myogenic cells displayed cytoplasmic confluence with such stumps (Fig. 8).

An extremely important observation in this study was the fusion of a myogenic cell located beneath the external lamina with an apparently undamaged segment of parent myofibre at a considerable distance from the site of injury (Fig. 9). This myogenic cell contained evidence of sarcomere production and cytoplasmic continuity was evident at multiple sites between it and the adjacent myofibre (Fig. 10 & 11).

All the observations indicate that the fusion of myogenic cells with each other, with the stump site of resealed fibres and with the undamaged parts of the injured fibre are the means by which the zones of necrosis are bridged, resulting eventually in the reconstitution of the myofibre.
For myoblast transfer therapy to be effective, donor myoblasts need to incorporate into myofibres. Detailed knowledge of myogenic fusion is therefore critical in understanding the efficiency that can be expected from fusion of injected donor myoblasts and regenerating myofibres. The observations that fusion of mononuclear and multinuclear myogenic cells occurs at the end and periphery of sarcoplasmic stumps indicates that this will be an important site of incorporation of injected donor myoblasts. Furthermore at such sites of segmental necrosis the donor myoblasts can probably traverse the damaged external lamina. It is not known, however, whether donor myoblasts can traverse the intact external lamina and occupy a satellite cell position in the undamaged areas of host myofibres. However if they can, then fusion of normal donor myoblasts might also occur at several sites along the length of damaged or undamaged segments of host myofibres (as documented in this report) resulting in a much more widespread introduction of donor myoblasts into dystrophic myofibres than would otherwise occur.

FIG.1. Numerous vesicles (arrows) in an area of close apposition of two myotubes from a 4 day chemically injured animal. Several fusion sites can be observed (asterisk), x 31,200.
FIG. 2. A Golgi region near the closely apposed membranes of two myotubes (4 day chemically treated animal), x 26,000.

FIG. 3. Electron micrograph of two fused myotubes in muscle sampled 4 days after chemical injury. An intermediate junction (arrow) can be seen and cytoplasmic confluence can be observed between the fused myotubes (asterisk), x 36,000.
FIG. 4. Light micrograph of a sarcoplasmic stump from a 4 day chemical induced injury. Organelles are sparse and a 5 myonuclei can be observed in the sarcoplasm, x 612.

FIG. 5. A elongated sarcoplasmic stump from a 4 day chemically injured animal. Myotubes and myoblasts can be observed in close apposition to the process in this light micrograph (arrow), x 372.
FIG. 6. Myogenic cells can be seen in close apposition to the sarcoplasmic stump from figure 5, x 660.

FIG. 7. An electron micrograph of a sarcoplasmic stump similar to that shown in figure 5. A myoblast can be seen in close apposition to the plasma membrane of the bud (arrow), x 1,600.
FIG. 8. A high power electron micrograph of a region of possible fusion from figure 7. Although plasma membranes of stump and myoblast can be observed in one area (solid arrows) cytoplasmic confluence can be seen in another region (hollow arrows), x 13,200.

FIG. 9. A myogenic cell located between the external lamina and plasmalemma of a myofibre (i.e. in the satellite cell position). Two areas of cytoplasmic confluence between these two opposed cells were seen (arrows), x 13,200.
FIG. 10. High magnification of an area of cytoplasmic continuity between the opposing cells in figure 9. Sarcomeric structures can be seen in the cytoplasm of this cell (hollow arrow). Cytoplasmic confluence indicative of fusion can be observed between the two cells (solid arrow), x 42,000.

FIG. 11. Another probable fusion site of cells in figure 9 where cytoplasmic confluence is apparent and the opposing membranes are distinctive (arrow). x 43,000.
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REFERENCES


DISCUSSION

Professor George Karpati:
Henning Schmalbruch of Copenhagen has found that one of the prerequisites for the fusion of intracellular vesicles with the surface membrane from within, during exocytosis, was that a patch of the membrane be clear at the site of intramembranosus particles. If the intramembranosus protein particles are sticking out, the 2 membranes cannot come together. He used a chemical, chlorheterine which has enhanced the formation of these "bare" patches that were clear of intramembranosus proteins which enhanced exocytosis. Do you think that in the fusion process, which you have described in the various cell types, it is a prerequisite too?

Dr. Terry A. Robertson:
I am very sure that is the case. I have read that article mentioned. I have not looked at intramembranosus particles in my work, but I would assume they certainly would be cleared at the site of fusion.

Professor George Karpati:
Have you got any speculations on the mechanism of the disposal of all that membrane. Does it just go away?

Dr. Terry A. Robertson:
The vesicles?

Professor George Karpati:
That the redundant membrane is ejected.
Dr. Terry A. Robertson:
With vesicles travelling backward and forward to the outside, I am sure that both the vesicles and redundant plasma membranes of the cells following fusion would be recycled through the Golgi region. Cytoplasmic movement is occurring continuously and I am sure that there would not be any problems of clearing these structures. Once fusion has occurred these structures disappear.

Dr. Edna C. Hardeman:
In the last illustration how do you know that the cell in the satellite cell position is not separating from the myofibre, and not the other way around?

Dr. Terry A. Robertson:
I am sure this is not the case. I have read an article by Schultz (1976) where he looked at growing muscle from ice and he presented an electron micrograph (Fig 8) which he interpreted as fusion of a satellite cell with a myofibre. Very large vacuoles separated the two cells and I would not interpret this as satellite cell fusion. To me fusion only occurs when two plasma membranes are in extremely close apposition. The cell I have described displayed sarcomeric differentiation and fusion where the myofibre would contribute new myonuclei and myofilaments to the sarcoplasm of the myofibre.

Professor Byron A. Kakulas:
Terry, in foetal development there is splitting off, of populations of muscle fibres, and this is one way hyperplasia develops. The same thing happens in mature animals under conditions of stretch, that is splitting off occurs and new fibres form. I think you have mentioned everything except fusion of myofibres to myofibres. Is it true that that doesn't happen?

Dr. Terry A. Robertson:
I certainly have not seen fusion of myofibres to myofibres. I have stated before, that myogenic fusion occurs within minutes. I am certain that the reason that a thorough study of myogenic fusion has not been carried out is that it needs patience and considerable time to cut the hundreds of blocks and examine hundreds of grids to find each particular fusion event or each myogenic cell and even then there is no guarantee that you will find it. It may happen but I certainly have not seen it yet. The cell in the satellite cell position fusing with the myofibre was only photographed 4 weeks ago. It is an exciting find when viewing the screen of an electron microscope to view such an event. To my knowledge this has certainly not been reported in the literature before.

Dr. Terence A. Partridge:
You showed a myoblast and a cell which was fusing. Are you sure that it was a single cell and not a myotube?

Dr. Terry A. Robertson:
No, I do not think that it was a myoblast. If we compare its size with that of an activated satellite cell next to it, it is a much larger cell. I think that it is an odd cut through a myotube as I have often seen myotubes containing 4 or 5 nuclei in an identical position. I think that satellite cells can be stimulated in normal areas of a myofibre to proliferate and fuse with each other. The resulting myotube can then be induced to fuse with the myofibre.