M-cadherin is a Reliable Marker of Quiescent Satellite Cells in Mouse Skeletal Muscle

Anton Wernig, Michael Bone[(1)], Andrey Irintchev, Ralf Schäfer, and Michael J. Cullen[(1)]

Department of Physiology, Neurophysiology, University of Bonn, Wilhelmstrasse 31, Bonn, Germany and (1) Department of Neuroscience, The Medical School, University of Newcastle, Framlington Place, Newcastle upon Tyne, United Kingdom

Abstract

Satellite cells are responsible for growth, maintenance and repair of postnatal skeletal muscle. On the search for a specific marker molecule for the identification of satellite cells by light-microscopy, we examined M-cadherin, a Ca^{2+}-dependent cell adhesion molecule which is present in quiescent as well as activated satellite cells but not in other cell types in skeletal muscle. To determine the frequency of M-cadherin positive satellite cells in relation to total satellite cells in intact adult mouse muscle, we evaluated every satellite cell found in spaced serial sections of single muscle fibre preparations immunostained for M-cadherin by light and electron microscopy.

We could identify 50 quiescent satellite cells this way and found M-cadherin antibody label in all of them. Statistically, this indicates that 94-100% of all satellite cells express M-cadherin, thus rendering M-cadherin as a reliable marker of quiescent satellite cells in mouse muscle.

Key words: M-cadherin; cell marker; myogenesis; satellite cells; skeletal muscle.

Basic Appl Myol 14(3): 161-168, 2004

The generation of skeletal muscle tissue is not restricted to embryonic development but continues throughout life during episodes of tissue growth or repair. Post-natal myogenesis is brought about by mononucleated precursors called satellite cells (for a review see [36]). During muscle growth, satellite cells divide and supply nuclei to the enlarging myofibres which contain only division-incompetent post-mitotic nuclei [25, 34]. After cessation of muscle growth, satellite cells become mitotically quiescent [35] but retain the ability to re-enter rapidly the mitotic cycle as soon as muscle fibre enlargement (hypertrophy) or repair (regeneration) are required [23, 26]. Activated satellite cells give birth to daughter cells called myoblasts which, after several replication cycles, mature and fuse predominantly with either pre-existing fibres (growth) or with each other (regeneration) [34]. Other daughter cells, in contrast, withdraw early from cycling and settle as new quiescent precursors beneath the basal lamina ensuring further self-renewal and regenerative potential. This dual behaviour is still present and most apparent in the progeny of cultured satellite cells and in expanded primary muscle cultures, from which muscle tissue and satellite cells are formed in vitro or at ectopic sites in vivo [3, 15].

Identification of satellite cells prior to the use of marker proteins had to be made by structural criteria resolvable only by electron microscopy. According to Mauro [22]: these are mononucleated cells located between the sarcolemma and the basal lamina of muscle fibres. This definition does not imply, however, that subendomysially located cells are a homogeneous cell population. On the contrary, a number of phenotypically distinct cell types (quiescent progenitors, activated satellite cells, replicating and differentiating myoblasts) reside beneath the basal laminae of muscle fibres during growth and repair (for a recent review see [12]). Furthermore, the pool of "quiescent" cells itself appears to be heterogeneous, including a majority of rapidly dividing precursors and a minority of slowly replicating cells of stem cell character [5, 34]. Finally, skeletal muscle appears to contain rare pluripotent cells of unknown identity and localisation [13, 17, 21], some of which, however, according to recent evidence, might be of hematopoetic origin [18].

Muscle precursors at different developmental stages (activated, proliferating, differentiated or maturing) can be identified by analyses of a variety of marker proteins [12]. However, for quiescent satellite cells, only few putative markers have been suggested: M-cadherin [6, 9, 16], c-met [8], myocyte nuclear factor [11], CD34 and Myf5 [4, 7], Pax7 [37] and NCAM [19]. A major problem concerning these markers is lack of definitive data as to whether they are expressed in all or in a fraction of the satellite cell pool only. In this study we analysed, at the electron- and light-microscopic level, whether satellite cells identified by morphological criteria are also labelled by a polyclonal antibody raised against M-cadherin. To avoid false negative results due to penetration problems in tissue blocks, we used enzymatically-dissociated whole single fibres of adult mouse muscles containing satellite cells and an intact basal laminae. The results of this study identify M-cadherin as a reliable marker for all quiescent satellite cells in mouse muscle.

Materials and methods

Fibre preparation

Individual fibres were explanted from the superficial portions of 4 tibialis anterior muscles of 2 male C57Bl/10 mice (8-week-old, Charles River Deutschland, Sulzfeld, Germany) as described previously [29, 30]. The muscles were incubated in collagenase type I (Sigma, Deisenhofen, Germany) at 37°C for 1.5 hours and then liberated by repeated pipetting with a wide-mouth Pasteur pipette. Immediately thereafter, the fibres were fixed in 4% paraformaldehyde in PBS at 37°C for 5 minutes, washed in PBS and then permeablised by incubation in 0.3% Triton X-100 (Sigma) in PBS for 5 minutes. Following another wash in PBS, the fibres were incubated in 20% normal goat serum (Jackson ImmunoResearch Laboratories, distributor Dianova, Hamburg, Germany) for 30 minutes at room temperature to reduce non-specific binding of antibody. Immunostaining for M-cadherin was performed by suspending the fibres in 0.7% lambda carrageenan (a non-gelling form of a vegetable gelatine, carrageenan type IV, Sigma) containing antibody at optimal dilution (1:50) for 20 hours at 4°C, followed by incubation with a horseradish peroxidase-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) for 1.5 hours at room temperature. The affinity-purified polyclonal antibody against M-cadherin was raised in rabbits as described previously [28] and its specificity was tested by Western blotting, ELISA and immunohistochemistry, which showed no cross-reactivity with other adhesion molecules (NCAM, N-cadherin and E-cadherin) [16]. The fibres were then immersed in peroxidase substrate solution containing diaminobenzidine (0.5mg ml^{-1}, Sigma) and hydrogen peroxide (0.02% v/v) in PBS buffer for 10 minutes at room temperature. Following this the fibres were washed, post-fixed for 30 minutes in osmium tetroxide (1% w/v) in 0.05M phosphate buffer and embedded in synthetic resin (Epon, Roth, Karlsruhe, Germany).

Electron microscopy

The polymerised resin was cut into blocks that could be orientated in the microtome (Reichert Ultracut E) so that the fibres were sectioned as near transversely as possible. Sections were sampled at intervals of 10 µm. At each sampling point two serial 1 micron sections were cut, one of which was kept unstained while the other was stained with toluidine blue to check for the presence of myonuclei and/or satellite cell nuclei. If, under the light microscope, nuclei could be detected, ultrathin 80nm sections were then cut off the same block face. Some of these sections were left unstained while others were stained with uranyl acetate and lead citrate. Thus, four types of sections could be compared and cross-related: (1) 1 micron unstained, (2) 1 micron stained, (3) ultrathin unstained and (4) ultrathin stained. When, under the electron microscope (Philips CM100) a satellite cell was detected in an ultrathin stained section from its position between the basal lamina and the plasma membrane of the myofibre, an unstained section of the same cell was examined to determine whether it displayed the electron-dense reaction product of the peroxidase reaction following immunostaining for M-cadherin. Care was taken that, due to the narrow spacing of the sample (10µm), a satellite cell nucleus was counted only once.

Results

Figure 1a shows a satellite cell on an isolated muscle fibre labelled with M-cadherin antibody and visualised with a peroxidase-conjugated secondary antibody. Such stained fibres were embedded in Epon and sectioned in a spaced-serial way at distances of 10µm. Semithin (1.0µm) sections were stained with toluidine blue to identify myonuclei (Figs 2a, 2b). All nuclei at the edge of a muscle fibre were consequently identified on the adjacent ultrathin sections (unstained or stained with uranyl acetate and lead citrate, Figs 2c, 2f and 2d, 2e respectively) in the electron microscope. In case of positive identification as a satellite cell on structural grounds, i.e. position external to the plasma membrane of the myofibre and internal to the basal lamina (Figs 2d-f), the unstained adjacent serial section was searched for peroxidase reaction product (Fig. 2c and 2f). The electron-dense product of the peroxidase reaction was most concentrated towards the edge of the cells (Fig. 2d) but was often seen throughout the cytoplasm (Fig. 2e). The reaction product was also seen in the narrow gap between the satellite cell and myofibre as well as in those areas of the myofibre cytoplasm that faced a satellite cell (Figs 2d-f).

The necessity of the electron microscope for the identification of satellite cells without further label is illustrated in Fig. 3a; in the light microscope, the nucleus at the lower edge resembles a satellite cell nucleus because myofibrils in its vicinity are missing. Under the electron microscope, however, it is clear that the nucleus lies inside the myofibre plasma membrane rendering it a myonucleus (Fig. 3b). On the opposite side of the fibre there is a small cell process which, from its position between the plasma membrane and basal lamina, is most likely the process of a satellite cell (its nucleus being out of the plane of the section) (Fig. 3c). This small profile is heavily labelled with peroxidase reaction product.

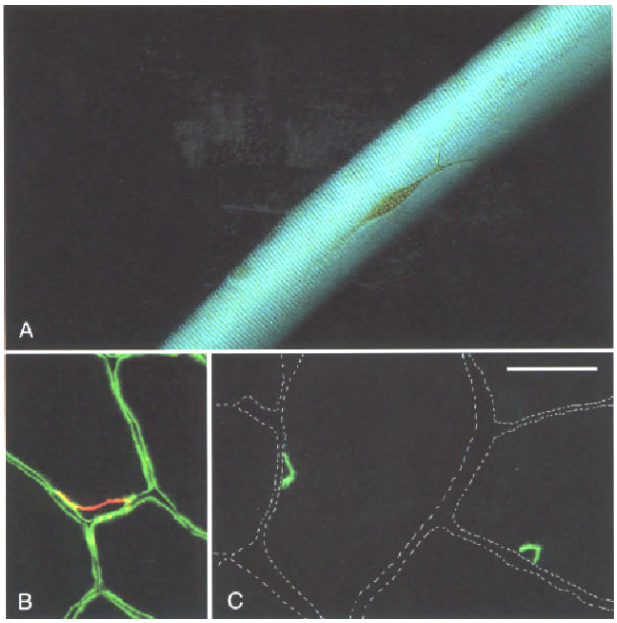

Figure 1: Light-microscopic visualisation of mouse (a), human (b) and rat (c) satellite cells.
a: Peroxidase reaction product (brown) after immunostaining of a single muscle fibre for M-cadherin delineates a satellite cell with spindle-shaped cell body and slender cell processes at both poles. The muscle fibre has been enzymatically and mechanically dissociated from the tibialis anterior muscle of an adult C57Bl/10 mouse.
b and c: Immunofluorescence staining for M-cadherin (red in b, green in c) in transverse cryostat sections from m. vastus lateralis of a 12-year old boy (b) and m. soleus of an adult rat (c). In b, an additional immunolabelling for laminin (green) delineates the basal laminae of muscle fibres. Note that the M-cadherin positive cell (prominent red staining at the site of apposition of the plasma membranes of the satellite cell and the myofibre) is located inside the basal lamina of the muscle fibre (green). Similar localisation at the opposing plasma membranes is seen in two satellite cells in the section of the rat muscle (c). Nuclear staining (BisBenzimide, not shown) was present in all three satellite cell profiles shown in b and c. Detection of the primary antibodies (M-cadherin, laminin) was done using a DTAF (green) or a rhodamine-conjugated (red) secondary antibody. Muscle fibre sarcolemma are outlined in white dashes. Scale bar = 40µm for a and 20µm for b and c.

A second example of a labelled satellite cell process is shown in Figs 3d and 3e. Note the absence of peroxidase label around the myonucleus in the same field (Fig. 3d). A myonucleus may be located adjacent to a satellite cell (from which it may have been derived) (Fig. 3f); in such a case the labelling product is also present close to the myonucleus where it faces the satellite cell.

Using this accurate examination procedure, 50 satellite cells were examined. All were positive for M-cadherin, none were encountered without the distinguishing peroxidase reaction product. No reaction product was seen associated with myonuclei except when adjacent to a satellite cell as described above. Importantly, each satellite cell identified ultrastructurally was found to be positive for M-cadherin in the adjacent unstained semithin section. The label was generally more clearly discernible from background in the light-microscopie than in the electron microscope (see Figs 2c and 2f). Even better signal-to-noise ratio can be achieved when using immunofluorescence technique to visualise quiescent satellite cells in cryostat sections of mouse (not shown, [16]), human (Fig. 1b) and rat muscles (Fig. 1c).

No evaluation of the satellite cell to myonucleus ratio was attempted since only transverse sections were carried out and the relative lengths of the two types of nuclei could not be determined. The latter would be necessary, however, in order to evaluate the relative probabilities of sampling the two types of nuclei in a thin section [1, 40].

Discussion

This study investigates how many of the satellite cells present in normal mouse muscle are associated with M-cadherin protein. The number of satellite cells (normalised per number of muscle fibre profiles or myonuclei) in sections or on isolated single fibres immunostained for M-cadherin in this study were within the percentage ranges found in previous studies carried out in our lab with fine-structural analyses [16, 27, 31]. Statistically, the observed proportion of labelled cells (100%, sample size 50) allows the prediction that 94-100% of all quiescent satellite cells are M-cadherin-positive (95%-confidence intervals for proportions, [33]). The clear conclusion is that M-cadherin is present in most quiescent precursor cells. We still cannot exclude, however, that a small fraction of the satellite cell pool is M-cadherin-negative as claimed on indirect evidence by Beauchamp et al. [4].

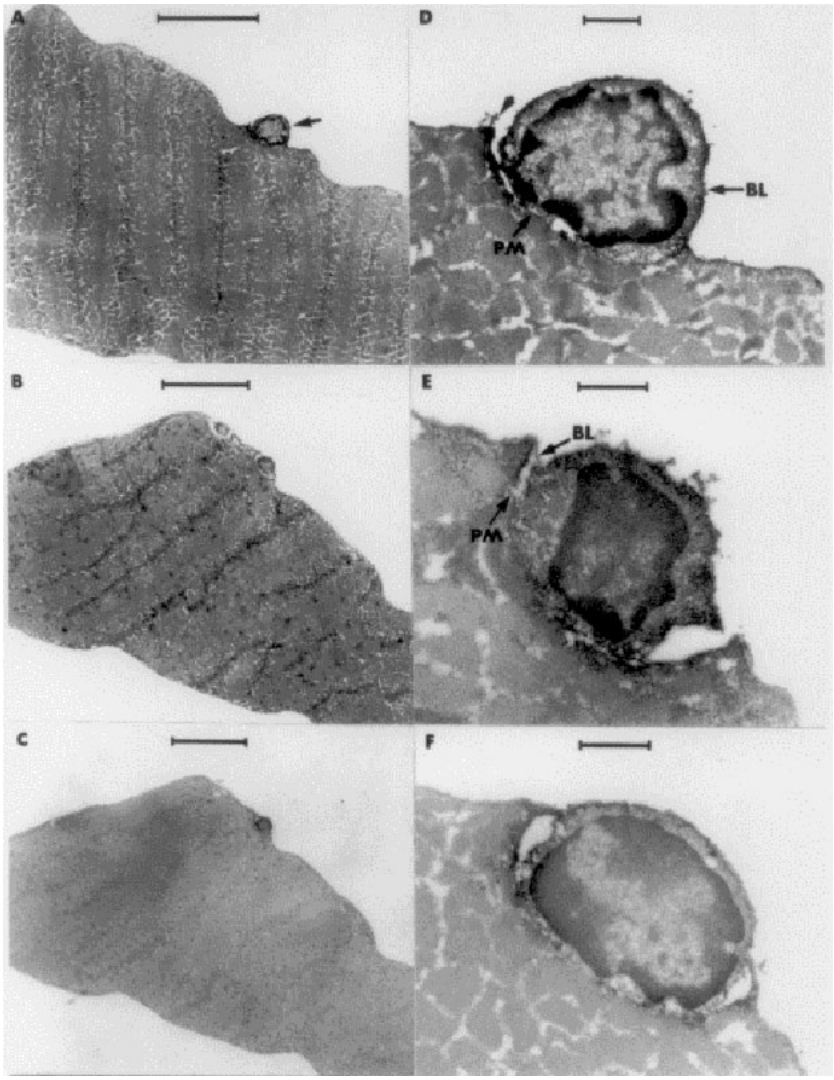

Figure 2. a: Transverse toluidine blue-stained 1.0µm Epon section through a single myofibre of mouse tibialis anterior muscle immunostained for M-cadherin using a peroxidase-conjugated secondary antibody. The reaction product makes the cell (arrow) on the right hand side of the fibre conspicuous. Scale bar = 10µm. Magnification = x 2,000.

b: Transverse section of a myofibre treated in the same way as that in (a). In this case the reaction product on the presumed satellite cell is not so prominent. Scale bar = 10µm. Magnification = x 1,600.

c: Transverse section of the same fibre as that seen in (b) approximately 1.0µm further along the fibre but not stained with toluidine blue. This allows the peroxidase reaction product to be seen more clearly than in b. Scale bar = 10µm. Magnification = x 1,500.

d: Electron micrograph of the cell highlighted in (a). Its position between the basal lamina (BL) and plasma membrane (PM) of the myofibre distinguishes it as a satellite cell. Part of the basal lamina (arrowhead) on the left hand side of the cell is fractured. Electron dense peroxidase reaction product is seen in the cytoplasm of the cell, more concentrated towards its periphery. Section stained with uranyl acetate and lead citrate. Scale bar = 1.0µm. Magnification = x 11,000.

e: Electron micrograph of the cell seen in (b). This cell is also positioned between the basal lamina (BL) and plasma membrane (PM) of the myofibre. It also contains peroxidase reaction product which in this case is distributed throughout the cytoplasm. Section stained with uranyl acetate and lead citrate. Scale bar = 1.0µm. Magnification = x 13,000.

f: Same cell as (b), (c) and (e), the section being taken approximately 2.0µm further along the fibre than that seen in (e). The section has not been stained with either uranyl acetate or lead citrate. Reaction product can be seen in the cytoplasm of the satellite cell and at the edge of the myofibre where it faces the cell. Scale bar = 1.0µm. Magnification = x 13,000.

The reliability of M-cadherin as a molecular marker of satellite cells has been questioned by other experimental evidence [8]. The authors have studied mononucleated cells, assumed to be satellite cells because of their association with explanted fibres and have used reverse-transcriptase polymerase chain reaction to monitor M-cadherin mRNA expression at single-cell level without simultaneous examination of protein expression

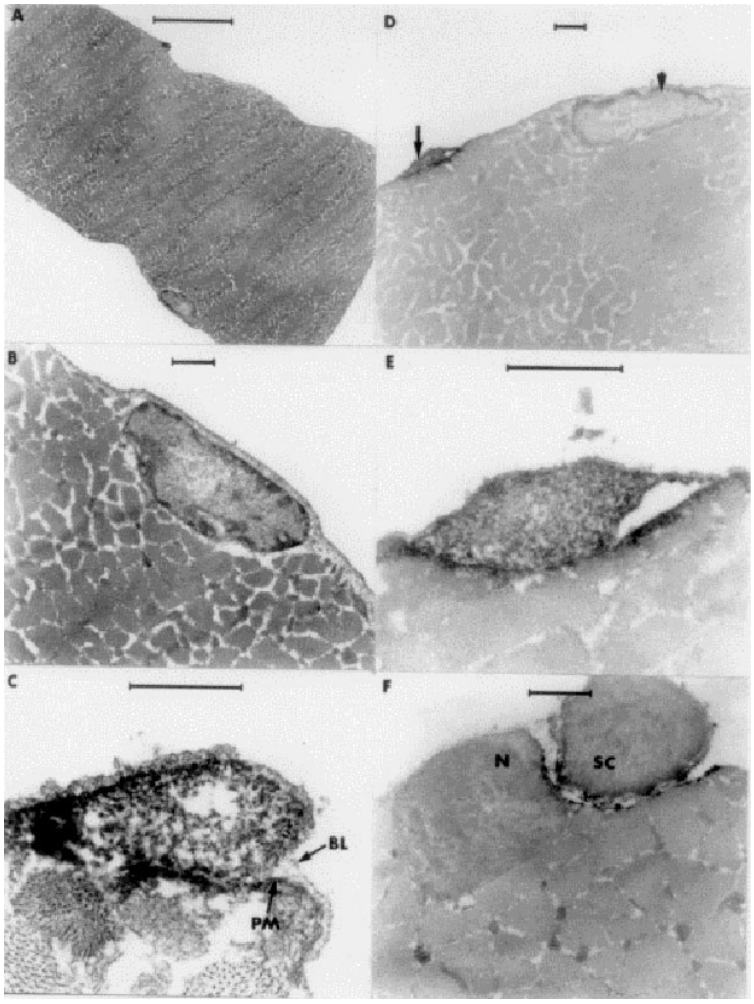

Figure 3. a: Transverse toluidine blue-stained 1.0µm Epon section through a single myofibre of mouse tibialis anterior muscle immunostained for M-cadherin using a peroxidase-conjugated secondary antibody. Scale bar = 10µm. Magnification = x 1,700.

b: Electron micrograph of the myonucleus seen at the lower edge of the fibre in (a). Scale bar = 1.0µm. Magnification = x 8,000.

c: Another part of (a) viewed at higher magnification. The cell process is interpreted as being the tail of a satellite cell from its position between the basal lamina (BL) and plasma membrane (PM) of the myofibre. The cell cytoplasm contains electron dense peroxidase reaction product. Scale bar = 1.0µm. Magnification = x 25,000.

d: Low power electron micrograph of an unstained transverse section through a fibre of mouse tibialis anterior muscle that has been immunolabelled for M-cadherin. To the left (arrow) is a cell process made conspicuous by the electron dense reaction product and to the right (arrowhead) is a myonucleus. Scale bar = 1.0 µm. Magnification = x 6,000.

e: Higher power electron micrograph of the cell process seen in (d). Scale bar = 1.0µm. Magnification = x 25,000.

f: Electron micrograph of an unstained section through a fibre that has been immunolabelled for M-cadherin. To the left is a myonucleus (N) and to the right a satellite cell (SC). Reaction product is only seen near the myonucleus where it faces the satellite cell. Scale bar = 1.0µm. Magnification = x 12,000.

however. The results show that only a small fraction (<20%) of the examined cells express M-cadherin mRNA immediately after fibre explantation. This observation has been interpreted to mean that M-cadherin is unsuitable as a molecular marker of quiescent satellite cells. However, our present results based on protein detection suggest that at least 94% of the quiescent satellite cells are M-cadherin positive. This apparent discrepancy is readily resolved if we assume that translational activity of the M-cadherin gene in quiescent cells is low or absent, and the protein has a considerable life time. Other gene expression studies have shown similar disparities between the expression levels for protein and mRNA [10, 38, 39]. The low or missing M-cadherin mRNA translational activity in quiescent satellite cells is enhanced after cell activation in vitro as indicated by the rapid increase in the proportion of positive cells (from <20% up to 100% within 96 hours; [8]). This observation correlates with the enhancement of mRNA and protein expressions after muscle injury in vivo when satellite cells become activated for myogenesis [16, 24].

The M-cadherin immunostaining of quiescent satellite cells on cross-sections of skeletal muscle is typically confined to the site of apposition of the plasma membranes of satellite cell and muscle fibre resulting in characteristic short (<10µm) and thin arc-shaped label at the level of the cell body (nucleus) (Figs 1b and 1c, [16]). Outside the nuclear region (cell processes, see Fig. 1a) even smaller profiles are recognisable. On longitudinal sections, mostly elongated, spindle-shaped profiles are seen indicating that most, though not all, satellite cells are oriented in parallel to the muscle fibres (see Fig. 1a). It is obvious that this antibody also labels quiescent satellite cells in other species than mouse, like human and rat (Figs 1b and 1c). We do not know, however, whether nearly all satellite cells carry the protein in these species. In fact, considering the much longer life span and the supposedly low level of mRNA in quiescent cells [8] it would be surprising to find high levels of protein in all human satellite cells quiescent for many years lest there is intermittent upregulation. We might not know all circumstances that lead to upregulation of the protein; however, we have seen upregulation of M-cadherin when satellite cells start to proliferate and myoblasts are incorporated e.g. with damage of muscle fibres [16]. Other situations might be postnatal fibre growth (when incorporation of proliferated satellite cells is prominent and causes a not well understood drop in their numbers, s. [34]) and hypertrophy, but also during recovery from atrophy (which supposedly goes along with loss of myonuclei, see [2, 14]). If all these situations were to occur, then activation of satellite cells might take place more often during a human life span than assumed. In fact, indirect evidence from the remaining growth capacity of satellite cells derived from biopsies of differently aged donors indicates that in the average each satellite cell divides 2 times in a 10 years life span [20]. Such events might prevent the complete loss of M-cadherin protein from the aged human satellite cell and render M-cadherin as a marker also for quiescent satellite cells in humans [32]. It will be of interest to obtain quantitative data on the expression of other putative markers of quiescent satellite cells such as c-met, myocyte nuclear factor, CD34 and Myf5 [4, 8, 11]. This will improve our understanding of the biology of skeletal muscle precursor cells and the putative pluripotent stem cells residing amongst them.

Conclusion

This study provides direct evidence that the vast majority of muscle satellite cells in mouse muscle can be identified by immunolabelling with an antibody raised against M-cadherin.

Acknowledgements

Dr. J. David Rosenblatt, while a Fellow of the Humboldt Foundation at the Department of Physiology of the University in Bonn, performed the single fibre preparations, M-cadherin staining and embedding of the material. The work was supported by a grant from The European Commission (BMH4-97-2767) to AW and MJC.

Address correspondence to:

Anton Wernig, Department of Physiology, Neurophysiology, University of Bonn, Wilhelmstraße 31, D-53111 Bonn, Germany; phone: +49 228-287-2274, fax: +49 228-287-2208, E-mail: a.wernig@uni-bonn.de.

References

[1] Abercrombie M: Estimation of nuclear population from microtome sections. *Anat Rec* 1946; 94: 239-247.

[2] Allen DL, Monke SR, Talmadge RJ, Roy RR, Edgerton VR: Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibers. *J Appl Physiol* 1995; 78: 1969-1976.

[3] Baroffio A, Hamann M, Bernheim L, Bochaton-Piallat ML, Gabbiani G, Bader CR: Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells. *Differentiation* 1996; 60: 47-57.

[4] Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS: Expression of CD34 and myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J Cell Biol* 2000; 151: 1221-1234.

[5] Beauchamp JR, Morgan JE, Pagel CN, Partridge TA: Dynamics of myoblast transplantation reveal discrete minority of precursors with stem cell-like

properties as the myogenic source. *J Cell Biol* 1999; 144: 1113-1122.

[6] Bornemann A, Schmalbruch H: Immunocytochemistry of M-cadherin in mature and regenerating rat muscle. *Anat Rec* 1994; 239: 119-125.

[7] Cooper RN, Tajbakhsh S, Mouly V, Cossu G, Buckingham M, Butler-Browne GS: In vivo satellite cell activation via Myf5 and MyoD in regenerating mouse skeletal muscle. *J Cell Sci* 1999; 112: 2895-2901.

[8] Cornelison DD, Wold BJ: Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. *Dev Biol* 1997; 191: 270-283.

[9] Donalies M, Cramer M, Ringwald M, Starzinski-Powitz A: Expression of M-cadherin, a member of the cadherin multigene family, correlates with differentiation of skeletal muscle cells. *Proc Natl Acad Sci USA* 1991; 88: 8024-8028.

[10] dos Remedios CG, Berry DA, Carter LK, Coumans JV, Heinke MY, Kiessling PC, Seeto RK, Thorvaldson T, Trahair T, Yeoh T, Yao M, Gunning PW, Hardeman E, Humphery-Smith I, Naidoo D, Keogh A: Different electrophoretic techniques produce conflicting data in the analysis of myocardial samples from dilated cardiomyopathy patients: protein levels do not necessarily reflect mRNA levels. *Electrophoresis* 1996; 17: 235-238.

[11] Garry DJ, Yang Q, Bassel-Duby R, Williams RS: Persistent expression of MNF identifies myogenic stem cells in postnatal muscles. *Dev Biol* 1997; 188: 280-294.

[12] Grounds MD: Muscle regeneration: molecular aspects and therapeutic implications. *Curr Opin Neurol* 1999; 12: 535-543.

[13] Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC: Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 1999; 401: 390-394.

[14] Hikida RS, Van Nostran S, Murray JD, Staron RS, Gordon SE, Kraemer WJ: Myonuclear loss in atrophied soleus muscle fibers. *Anat Rec* 1997; 247: 350-354.

[15] Irintchev A, Rosenblatt JD, Cullen MJ, Zweyer M, Wernig A: Ectopic skeletal muscles derived from myoblasts implanted under the skin. *J Cell Sci* 1998; 111: 3287-3297.

[16] Irintchev A, Zeschnigk M, Starzinski-Powitz A, Wernig A: Expression pattern of M-cadherin in normal, denervated and regenerating mouse muscles. *Dev Dynam* 1994; 199: 326-337.

[17] Jackson KA, Mi T, Goodell MA: Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* 1999; 96: 14482-14486.

[18] Kawada H: and Ogawa M: Bone marrow origin of haematopoietic progenitors and stem cells in murine muscle. Blood 2001; 98: 2008-2013.

[19] Kadi F: Adaption of human skeletal muscle to training and anabolic steroids. Acta Physiol Scand Suppl. 2000; 646: 1-52.

[20] Knauf U*, Schäfer R*, Zweyer M, Högemeier O, Liu X, de Guarrini F, Wernig A: Exhaustion of the muscle organotypic stem cell with ageing. *equal contribution. *First International Meeting of the Stem Cell Network North Rhine Westphalia* 2002; p 12-13.

[21] Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, Usas A, Gates C, Robbins P, Wernig A, Huard J: Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. *J Cell Biol* 2000; 150: 1085-1100.

[22] Mauro A: Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 1961; 9: 493-495.

[23] McGeachie JK, Grounds MD: Initiation and duration of muscle precursor replication after mild and severe injury to skeletal muscle of mice. An autoradiographic study. *Cell Tissue Res* 1987; 248: 125-130.

[24] Moore R, Walsh FS: The cell adhesion molecule M-cadherin is specifically expressed in developing and regenerating, but not denervated skeletal muscle. *Development* 1993; 117: 1409-1420.

[25] Moss FP, Leblond CP: Satellite cells as a source of nuclei in muscles of growing rats. *Anat Rec* 1971; 170: 421-436.

[26] Rantanen J, Hurme T, Lukka R, Heino J, Kalimo H: Satellite cell proliferation and the expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. *Lab Invest* 1995; 72: 341-347.

[27] Reimann J, Irintchev A, Wernig A: Regenerative capacity and the number of satellite cells in soleus muscles of normal and mdx mice. *Neuromuscul Disord* 2000; 10: 276-282.

[28] Rose O, Rohwedel J, Reinhardt S, Bachmann M, Cramer M, Rotter M, Wobus A, Starzinski-Powitz A: Expression of M-cadherin protein in myogenic cells during prenatal mouse development and differentiation of embryonic stem cells in culture. *Dev Dynam* 1994; 201: 245-259.

[29] Rosenblatt JD, Lunt AI, Parry DJ, Partridge TA: Culturing satellite cells from living single muscle fiber explants. *In Vitro Cell Dev Biol Anim* 1995; 31: 773-779.

[30] Rosenblatt JD, Parry DJ, Partridge TA: Phenotype of adult mouse muscle myoblasts reflects their fiber type of origin. *Differentiation* 1996; 60: 39-45.

[31] Rosenblatt JD, Partridge, TA, Wernig, A: The number of myogenic cells on muscle fibers. *European Journal of Neuroscience* 1998; 10: 365.

[32] Sajko S, Kubinova L, Kreft M, Dahmane R, Wernig A, Erzen I: Improving Methodological Strategies for Satellite Cells Counting in Human Muscle during Ageing. *Image Anal. Stereol*. 2002; 21: 7-12.

[33] Sachs L (eds): *Angewandte Statistik, Planung und Auswertung, Methoden und Modelle. (Vol 4)* Berlin, Heidelberg, New York: Springer-Verlag, 1974, p 262.

[34] Schultz E: Satellite cell proliferative compartments in growing skeletal muscles. *Dev Biol* 1996; 175: 84-94.

[35] Schultz E, Gibson MC, Champion T: Satellite cells are mitotically quiescent in mature mouse muscle: an EM and radioautographic study. *J Exp Zool* 1978; 206: 451-456.

[36] Schultz E, McCormick KM: Skeletal muscle satellite cells. *Rev Physiol Biochem Pharmacol* 1994; 123: 213-257.

[37] Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA: Pax7 is required for the specification of myogenic satellite cells. *Cell* 2000; 102: 777-786.

[38] Tennyson CN, Shi Q, Worton RG: Stability of the human dystrophin transcript in muscle. *Nucleic Acids Res* 1996; 24: 3059-3064.

[39] Vater R, Young C, Anderson LV, Lindsay S, Blake DJ, Davies KE, Zuellig R, Slater CR: Utrophin mRNA expression in muscle is not restricted to the neuromuscular junction. *Mol Cell Neurosci* 1998; 10: 229-242.

[40] Watkins SC, Cullen MJ: A quantitative study of myonuclear and satellite cell nuclear size in Duchenne's muscular dystrophy, polymyositis and normal human skeletal muscle. *Anat Rec* 1988; 222: 6-11.