

Intercellular Adhesion in Developing and Adult Skeletal Muscle: Analysis of M-Cadherin

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Abstract

M-cadherin is a member of the family of calcium-dependent intercellular adhesion molecules, the cadherins. These are involved in the establishment of intercellular junctions and are associated with morphogenetic events. This article focusses on M-cadherin during skeletal muscle development emphasizing its pattern of expression, its interaction with catenins and cytoskeletal elements, and functional aspects. So far, expression and functional studies imply that M-cadherin plays a role during fusion of myoblasts to myotubes. Based on data from different groups, it is discussed whether M-cadherin is a marker for the complete satellite cell pool *in vivo* which would be helpful for studies of both, physiological and pathophysiological situations of skeletal muscle in development and in the adult. Finally, we describe the genomic structure of the murine M-cadherin gene which is the basis for further functional and regulatory studies *in vivo*.

Key words: M-cadherin, expression pattern, cytoskeleton, gene structure.

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Cadherins are a family of calcium-dependent cell adhesion molecules, that mediate cell cell interactions (for review see ref. [33]). Each of the members of the cadherin family shows a unique pattern of tissue distribution which is developmentally regulated. Functionally, cadherins mediate the establishment of intercellular junctions and are associated with morphogenetic events, such as the separation of cell layers, epithelial-mesenchymal cell transitions and condensation or dispersion of cell mass [3, 15, 18, 44, 62]. Several members of the cadherin family have been described in the mouse i.e., N-cadherin [37], P-cadherin [19], E-cadherin [54], VE-cadherin [28] and M-cadherin [11], the latter being subject of this review.

Most of the members of the cadherin family share a common protein domain structure and a high degree of amino acid sequence homology, in particular in the N-terminal part of the extracellular region and the cytoplasmic domain [15]. Mutational experiments as well as NMR and X-ray analyses have revealed that the extracellular domains of the cadherins mediate the homophilic intercellular adhesion [42, 45, 53, 58]. The cytoplasmic tails of the cadherins complex with the cytoplasmic proteins α -catenin, β -catenin and plakoglobin as has been shown e.g. for E-cadherin [1, 7,

17, 23, 39, 41, 46], N-cadherin [22, 25, 35] and M-cadherin [36].

Two distinct cadherin-catenin complexes have been identified, e.g. in epithelial cells, cardiac myocytes and skeletal muscle cells: one, consisting of the respective cadherin, β -catenin and α -catenin, the second complex containing plakoglobin instead of β -catenin [1, 7, 22, 23, 36]. In some cases, it could be shown that the cadherin-catenin complexes interact with cytoskeletal elements such as the actin -microfilaments and associated proteins [24, 63]. It has been indicated that α -catenin is able to directly crosslink the actin filaments, thereby providing a molecular link from E-cadherin to the actin cytoskeleton [52]. This is supported by analyses showing that α -catenin has significant amino acid sequence homologies to the actin-binding domains of vinculin [21, 40]. Alpha-actinin, an actin filament-crosslinking molecule might provide an additional connection from the cadherin complex to the cytoskeleton as has been indicated for N-cadherin-expressing embryonal lung fibroblasts [35, 43]. Thus, beside the actin filaments and some of its associated proteins, no other element of the cytoskeletal filament network has been identified to interact with cadherin

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catenin complexes. Fig. 1 shows a schematic representation of a cadherin and associated proteins.

This paper focusses on the analyses of M-cadherin in myogenic cells but not on N-cadherin which has also been implied to play a role in myogenesis [50]. In addition, we report on the isolation and characterization of the murine M-cadherin gene. For analysis of M-cadherin in the glomeruli of the cerebellum where its postnatal onset of expression correlates with the establishment of synaptic connections between mossy fibers and granule cell dendrites see ref. [2] and [56].

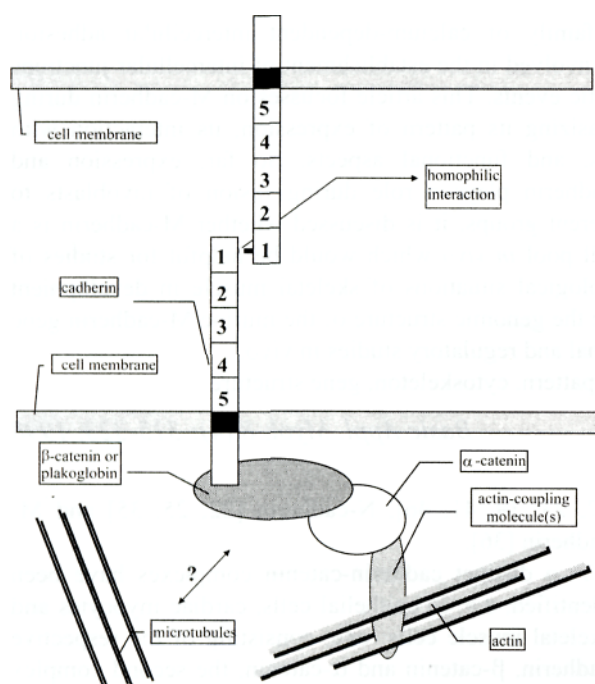


Figure 1. Model of cadherin interactions. Cadherins exert their intercellular adhesive properties via homophilic interaction of their extracellular domains (numbered 1 to 5). They have a single membrane spanning domain and are intracellularly associated via their cytoplasmic tail directly with β -catenin or plakoglobin. α -catenin is linked to β -catenin, or plakoglobin and these, directly or indirectly, to the actin cytoskeleton. For M-Cadherin, the molecular link of the cadherin-catenin complex to the microtubular filaments (see text) is not defined yet. M-Cadherin consists of 784 amino acids (aa) containing a signal-precursor-peptide and extracellular domain (aa 1-595), a transmembrane region (aa 596-625) and a cytoplasmic domain (aa 626 to 784). Further information about M-Cadherin can be obtained under GenBan[®] accession number M74541.

Expression Pattern of M-Cadherin in Prenatal and Postnatal Skeletal Muscle Cells

Prenatal skeletal muscle development

The pattern of expression of M-cadherin has been studied at the level of its mRNA by *in situ* hybridization and at the level of protein by immunofluorescence analysis with M-cadherin antibodies. Both types of investigations have revealed that M-cadherin is present in the somites. M-cadherin mRNA was first detectable at day 8.5 of embryonic development (E8.5) whereas M-cadherin protein was only visible at E10.1/3 in myotomal cells [38, 55]. *In situ* hybridizations and the immunofluorescence analysis were both performed on sections of the BALB/c mouse strain. This suggested that the temporal difference seen in the time of onset of M-cadherin mRNA and protein might indeed be a delay in M-cadherin translation or an increased M-cadherin protein turnover and not due to differences in genetic backgrounds.

After M-cadherin protein is turned on in myotomal cells, it continues to be expressed in skeletal muscle cells originating from somites [55]. During fetal development, M-cadherin changes its pattern of expression from a uniform surface distribution (until E13/E14) to the appearance of M-cadherin clusters as shown for E16 and E18. The occurrence of M-cadherin clusters coincides with the development of basal lamina structures as visualized by antibodies to laminin. M-cadherin and laminin labellings appear mutually exclusive. These observations suggest that M-cadherin determines the docking place for mononucleated myogenic cells on myotubes and myofibres where they become deposited as satellite cells during skeletal muscle development (see below).

Postnatal skeletal muscle development

In agreement with others, we found M-cadherin on satellite cells of intact muscle [5, 29, 51]. It is also expressed in myofibres beneath the satellite cells as indicated by immunoelectron microscopy studies [5]. In regenerating muscle, M-cadherin is upregulated in myoblasts and downregulated in the maturing myotubes implying a specific role for it in this process [29] which would be consistent with analyses of M-cadherin in terminally differentiating myoblasts [36, 48, 64].

Contradicting results have been obtained in terms of localization of M-cadherin at the neuromuscular junction. We and others [5, 29] could not detect M-cadherin at the neuromuscular junction whereas others [9] described its appearance on this structure. This discrepancy is still unresolved but might be caused by the use of different preparations of M-cadherin antibodies, for example anti-peptide antibodies as opposed to affinity purified antibodies raised against a

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fusion protein of M-cadherin and the prokaryotic maltose-binding protein [36, 55].

An important matter of debate is whether M-cadherin antibodies label all or only a fraction of the quiescent satellite cell population *in vivo*. An unambiguous answer to this question would be useful for *in vivo* studies of both physiological and pathological situations in skeletal muscle [29, 30, 31]. So far, satellite cells cannot be identified by biochemical means, e.g. by their pattern of cytoskeletal filaments [4]. Based on published data it has appeared that a high proportion of satellite cells in sections of intact skeletal muscle could be labelled by M-cadherin antibodies. In contrast to this assumption, Cornelison and Wold [10] claimed recently that only approximately 20% of satellite cells were M-cadherin positive. This result was based on mRNA analysis by RT-PCR of single cells isolated from single muscle fibre preparations which were kept in cell culture for 48 hours.

Most likely, the different estimations of M-cadherin positive satellite cells reflect differences in experimental set-ups. *In vivo*, satellite cells can be positively identified by their position between the sarcolemma and the basal lamina. Obviously, the positive identification of satellite cells is much more difficult in cell culture. Furthermore, factors regulating M-cadherin *in vivo* might be missing in cell culture, a situation which might lead to the picture of M-cadherin expression seen by Cornelison and Wold [10].

The following observation, however, is in favour of the idea that most if not all satellite cells *in vivo* are indeed M-cadherin positive. Previously, it has been shown by ultrastructural criteria that 4.6% of all myonuclei in a eight month old mouse soleus muscle are satellite cells [57, 59]. Soleus muscle of similar age contained a comparable number of myonuclei (4.2%) staining for M-cadherin which were also positively

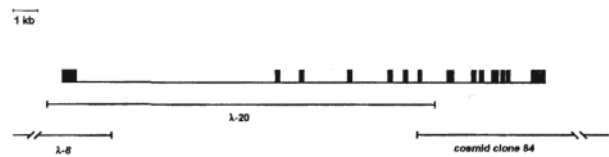


Figure 2. Genomic organization of the M-Cadherin gene. The M-cadherin gene consists of 14 exons and comprises a genomic region of 19.5 kb. A schematic alignment of fragments obtained from cosmid- (pcos4EMBL/ from A. Frischauf) and λ -phage mouse genomic libraries [49] is shown. Overlapping "k-clones 8 and 20 spans the 5-prime end with the putative promoter and exons 1-7 while the cosmid clone 64 harbours the exons 8-14. X8 and "k20 genomic clones were isolated with a 5' probe corresponding to bp 417-728 of the M-cadherin cDNA while clone 64 was obtained by screening with a probe spanning from bp 1495 to 3228 of the M-cadherin cDNA [Ace. No. M14541].

identified as satellite cells by their localization between the sarcolemma and the basal lamina ([51]; Wernig, personal communication). Based on this, it seems likely that M-cadherin is indeed a marker molecule for these cells in adult muscles.

M-Cadherin and its Association with Catenins and Cytoskeletal Proteins

One of the intriguing questions concerning cadherins is their mediation of cell type specific functions. For example, how much are these influenced by the amino acid composition (i.e. structure) of a given cadherin as opposed to cell type specific factors provided by a particular cellular environment (e.g. myogenic versus epithelial environment)? As mentioned earlier, cadherins commonly interact with the catenins to form two distinct complexes (Fig. 1). This has also been

Table I. Nucleotide sequences of exon-intron boundaries of the mouse M-cadherin gene.

Exon size (bp)	5' Splice donor	Sequence at exon-intron junctions	3' Splice acceptor	Intron size (kb)
561	CAG	gtaagct. .ctattgtgttttag	AGC	8.2
154	CAG	gtaagag ctccctccccacag	ATC	0.75
156	AGG	gtagggc...ctctgtttgttcag	CTA	1.75
145	CAG	gtgaggt. . . ctctgtcatccccag	GCA	1.45
161	GAG	gtgaggc. . . tcatattgtccccag	GTG	0.458
129	GAG	gtaccac. . . ggctgggactccccag	TTC	0.389
186	AAG	gtgagcc...gtcattcccctgcag	ccc	1.05
254	CAG	gtgggct...cctccccaccaatag	CTA	0.65
143	ATG	gtgaggg. . . tctcccttctccag	CCA	0.113
240	ACG	gtgcgct...gtgcgcctctacag	TGA	0.25
137	TAG	gtgagtg...ctctcgtgcctccag	TTC	0.098
165	CAG	gtaagga...ttactatgcacacag	GAT	0.113
575	GAT	gtaggtg...gggcttttccacag	GGC	1.0

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Table 2. Length of exons (bp) in different cadherin-genes.

Exon	N-cadherin	P-cadherin	E-cadherin	M-cadherin	VE-cadherin
1	392	116	175	561	56
2	112	118	121	154	221
3	227	74	224	156	288
4	147	126	144	145	117
5	156	156	156	161	165
6	144	145	145	129	188
7	174	176	176	186	251
8	138	129	129	254	143
9	186	186	183	143	125
10	254	241	245	240	109
11	143	146	146	240	246
12	234	225	225	137	209
13	234	210	228	165	
14	140	131	131	575	
15	165	147	144		
16	1481	883	1841		

Data were taken from the following references: 13, 37 (N-cadherin), 19 (P-Cadherin), 54 (E-Cadherin), 28 (VE-Cadherin), this paper (M-Cadherin).

Table 3. Intron length (in kb) of different mouse cadherin genes.

	N-cadherin	P-cadherin	M-Cadherin	VE-Cadherin
1	34.2	0.215	8.200	11.0
2	>106.25	23.0	0.750	>7.0
3	1.55	1.077	1.750	0.78
4	1.85	0.128	1.450	1.2
5	5.9	0.100	0.458	1.0
6	0.2	1.769	0.389	1.8
7	4.15	0.608	1.050	5.0
8	4.0	1.366	0.650	0.93
9	2.25	2.3	0.113	0.45
10	1.8	0.223	0.250	2.2
11	2.4	0.740	0.098	1.6
12	0.7	2.389	0.133	
13	2.1	4.559	1.000	
14	17.6	0.346		
15	16.0	2.224		

Data were taken from references indicated in the legend of table 2. With over 200 kb in length the N-cadherin gene is the longest among the cadherin genes. It is about 5 to 6 times longer than the P- (45 kb) the E- (40 kb) and the VE- (36 kb) cadherin genes and ten times longer than the M-Cadherin gene (20 kb).

demonstrated for M-cadherin in developing skeletal muscle cells in culture [36]. Thus, in terms of its interaction with the catenins, M-cadherin exhibits features similar to E-cadherin and other cadherins (see introduction) suggesting that the muscle specific function of the M-cadherin catenin complex might be mediated by interactions with yet unidentified cellular components.

Others have indicated that cadherin catenin complexes are associated, either directly or indirectly, with cytoskeletal elements, in particular the microfilament system (see introduction). Previous analyses of M-

cadherin in the *contactus adherens* of the synaptic glomeruli of the cerebellum raised, however, the possibility that M-cadherin might associate with cytoskeletal elements which differ from those connecting to the E-cadherin catenin complex in epithelial cells [56]. This is supported by co-immunoprecipitation and co-polymerization experiments from our laboratory demonstrating that the M-cadherin catenin complex can interact with microtubules in myogenic cells (Kaufmann U, Kirsch J, Irintchev A, Wernig A and Starzinski-Powitz A: 1998, submitted). When expressed in an ectopic

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environment such as epithelial cells, M-cadherin could not be co-precipitated with the microtubules. In turn, ectopical expression of E-cadherin in myogenic cells led to an interaction of this cadherin with the microtubules. In conclusion, our data are in favour of the idea that the myogenic environment provides a specific molecular link of the cadherin catenin complex to the microtubular system.

These findings are consistent with the model that the interaction of M-cadherin with microtubules might be essential to keep the myoblasts aligned during fusion, a process in which both, M-cadherin and microtubules have been implicated [26, 32, 64].

Involvement of M-Cadherin in Terminal Differentiation of Myoblasts

Previously, M-cadherin has been assigned to the cadherin family by its high degree of amino acid homologies to other cadherins, such as E-cadherin, N-cadherin and P-cadherin [11]. Expression of the full length cDNA of M-cadherin (Acc. No. M74541) in cadherin-negative mouse fibroblasts has shown that M-cadherin was able to induce a calcium-dependent, adhesive phenotype in these cells [36]. This also suggested that M-cadherin mediates its function by homophilic interaction.

Upregulation of M-cadherin during murine embryonic skeletal muscle development [55], muscle regeneration in the adult [29] and terminal myogenic differentiation in cell culture [11, 36, 48] suggested an important function in myogenesis. This assumption was supported by experiments in which the intercellular binding region of M-cadherin (and thus its function) was blocked

during terminal differentiation of skeletal muscle cells by addition of synthetic peptides from the putative binding region [64]. The block of M-cadherin by peptide led to a dose-dependent inhibition of myotube formation (myoblast fusion) but not of biochemical differentiation. This result is consistent with the idea that recognition of myoblasts as a prerequisite for fusion is, at least in part, mediated by M-cadherin. Obviously, N-cadherin which is also expressed in myogenic cells, is dispensable for the fusion process since N-cadherin null cells are still fusion competent [8].

Characterization of the M-Cadherin Gene

In order to understand the factors controlling the temporal and tissue specific expression of the M-cadherin gene, information about its genomic structure is necessary. In this paragraph we describe recombinant cosmids and λ - phages containing the murine full-size M-cadherin gene which has been assigned to chromosome 8 [33]. The probes used for isolation of the genomic clones are described in the legend to Fig. 2.

The exon-intron structure was determined by sequencing all genomic regions complementary to the M-cadherin cDNA and evaluating the exon boundaries by the breakpoint of homology and the consensus splice sequence. Analysis of these data showed that the M-cadherin gene consists of 14 exons ranging in size between 129 bp and 575 bp (Table 1) and spanning a genomic region of 19.5 kb (Fig. 2). The first exon encodes the 5'untranslated region and contains the codon for the putative initiator methionine (for details of the cDNA sequences see under Genbank accession

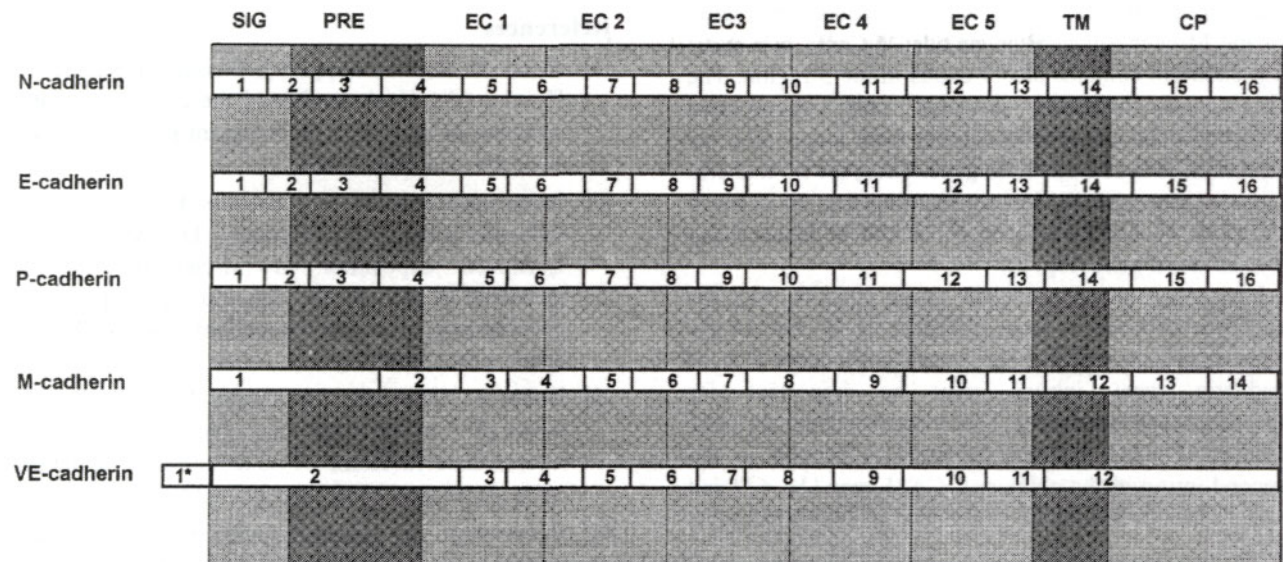


Figure 3. Comparison of the exon-length structure of N-cadherin [13, 37], P-cadherin [19], E-cadherin [54], M-cadherin (this paper) and VE-cadherin [28]. The exons indicated by rectangles are counted following previously published descriptions. The first exon (*) of VE-cadherin is untranslated. Abbreviations: SIG, signal peptide; PRE, precursor peptide; EC 1-5, extracellular domains 1 to 5; TM, transmembrane domain; CP, cytoplasmic domain.

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number M74541).

Intron lengths were determined by restriction fragment analysis and, where applicable, by exon-exon PCR (thereby amplifying short introns) and subsequent nucleotide sequence determination of the introns. The intron sizes are also summarized in Table 1. The first intron spreads over 8.2 kb whereas all the other introns were smaller and ranged from 113 bp to 1750 bp.

The M-cadherin gene encodes a single M-cadherin mRNA species. This was demonstrated by sequence determination and comparison of M-cadherin cDNAs from RNA of skeletal muscle cells and cerebellum (data not shown).

The nucleotide sequence of 250 bp upstream of the most 5' cDNA sequences of M-cadherin was determined (not shown). Analysis of these putative promoter sequences revealed a lack of conventional TATA and CAAT box consensus sequences as already shown for other cadherin genes [13, 54, 60].

Comparison of the M-cadherin gene structure with those of other cadherin genes

The size and structure of the M-cadherin gene was compared to those of the murine N-cadherin [37], E-cadherin [54], P-cadherin [19], and VE-cadherin [28] genes. Apart from the differences in size (see legend to Table 3), the genomic organization of these genes exhibit a similar genomic organization since exon-intron boundaries are, with two exceptions, very much conserved (Fig. 3).

The cytoplasmic region of E-, N-, P- and M-cadherin is encoded by three exons, whereas a single exon encodes the complete cytoplasmic domain of VE-cadherin. The N-terminal part of E-, N- and P-cadherin are encoded by three exons while the equivalent region of the M-cadherin gene is encoded by only one exon. VE-cadherin contains also 3 exons in the 5' region. The first exon, however, contains only untranslated sequences and the second exon is even larger than the first exon of the M-cadherin gene. Thus, the M-cadherin gene is composed of 14 exons in contrast to 16 exons in the in P-, N- and E-cadherin genes and 12 exons in the VE-cadherin gene (Fig. 3).

Like other mouse cadherin genes [19, 37, 54], M-cadherin harbours a large intron at the 5' site of the gene (Table 1 and 3). The consistently large size of cadherin gene introns may be important for transcriptional regulation. This is supported by previous studies showing that an enhancer is present in the second intron of the chicken L-C AM gene [16,61] and also in the second intron of the mouse P-cadherin gene [20].

E-cadherin, VE-cadherin, P-cadherin and M-cadherin have been mapped to the similar region of mouse chromosome 8 [12, 19, 28, 33]. This might reflect their

evolutionary origin from a common ancestor gene by duplication and diversification [47].

Conclusions

It has been indicated by several lines of evidence that M-cadherin is an intercellular molecule which plays a central role in the formation of skeletal muscle. In particular, M-cadherin is involved in the regulation of myoblast fusion. Another function might be that M-cadherin determines the place on myofibres where satellite cells will be deposited. Further studies, focussing on M-cadherin's function *in vivo* as well as on the signalling mechanisms mediated by M-cadherin and its interaction partners will now be required to fully understand the role of M-cadherin in skeletal muscle development.

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