Close encounters: regulation of vertebrate skeletal myogenesis by cell-cell contact

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Summary

Cells of the vertebrate skeletal muscle lineage develop in a highly ordered process that includes specification, migration and differentiation into multinucleated myofibers. The changes in gene expression and cell morphology that occur during myogenic differentiation must be coordinated with each other in a spatiotemporal fashion; one way that this might occur is through regulation of these processes by cell-cell adhesion and resultant signaling. The past several years have witnessed the identification of molecules that are likely to be mediators of the promyogenic effects of cell-cell contact and some of the mechanisms by which they work. These include: the community factor, embryonic fibroblast growth factor (eFGF); classical cadherins, which mediate both adhesion and signaling; and cadherin-associated immunoglobulin superfamily members such as CDO, BOC and neogenin. Genetic evidence for the promyogenic roles of some of these factors is emerging. In other cases, potential compensatory or redundant functions necessitate future construction of double or triple mutants. Mechanistic studies in vitro indicate that specific cadherins and immunoglobulin superfamily proteins exert some of their effects in an interdependent fashion by signaling from a multiprotein complex found at sites of cell-cell contact.

Key words: Muscle development, Cell adhesion, Cell differentiation, Signal transduction, Cadherin, Immunoglobulin superfamily

Introduction

In vertebrates, skeletal muscles of the trunk and limbs arise from somites, which develop from presomitic paraxial mesoderm and mature into distinct compartments. Muscle precursor cells are derived from the dorsal region of the maturing somite, the dermomyotome, and in turn form the myotome, a set of differentiated somitic muscle cells that eventually form the back musculature, or migrate to produce limb and other muscles (Ordahl et al., 2000; Tajbakhsh and Buckingham, 2000). Committed myoblasts must initially proliferate to produce appropriate numbers and then differentiate in a carefully orchestrated process that includes withdrawal from the cell cycle, expression of muscle-specific genes, morphological alterations and fusion to form multinucleated myofibers. The processes of myogenic specification and differentiation are coordinated by a family of myogenic basic helix-loop-helix (bHLH) transcription factors (Myf5, MyoD, myogenin and MRF4), which function in concert with transcription factors of the myocyte enhancer factor 2 (MEF2) family in a cell-autonomous positive-feedback network (Molkentin and Olson, 1996; Pownall et al., 2002; Yun and Wold, 1996). However, these processes are also regulated, at multiple steps, by extracellular signaling molecules, including diffusible factors, components of the extracellular matrix, and factors involved in cell-cell contact. Here, we review recent progress in the area of cell-cell contact in myogenesis, emphasizing the promyogenic functions of specific classical cadherins and immunoglobulin (Ig) superfamily members. Primary and secondary myoblast fusion, processes that by definition require cell-cell contact, have recently been reviewed elsewhere (Chen and Olson, 2004; Horsley and Pavlath, 2004) and are not discussed here in detail.

The community effect

The community effect is a phenomenon in which cells must contact a sufficient number of like neighbors if they are to undergo coordinate differentiation within a developing tissue (Gurdon et al., 1993a). It was first described by Gurdon and coworkers, who showed that a community of >100 explanted, gastrula-stage *Xenopus laevis* muscle precursor cells can interact in a contact-dependent fashion to enable muscle-specific gene expression and terminal differentiation, but smaller groups cannot (Gurdon, 1988; Gurdon et al., 1993b). The factors that mediate the community effect could be either associated with the cell surface or secreted and diffusible. In the latter case, a minimally sized community of cells in group contact would be required to achieve a sufficient level of the factor within the community. Strong evidence supports such a scenario in the case of *Xenopus* myogenesis. A member of the fibroblast growth factor family, eFGF (the *Xenopus* ortholog of FGF4), can function as a community signal in this system, supplanting the need for group contact and stimulating muscle-specific gene expression in single precursor cells (Standley et al., 2001). Furthermore, inhibition of FGF signaling prevents differentiation of aggregated precursors, and factors involved in earlier developmental processes important for myogenesis (e.g. mesoderm induction and patterning), such as Xwnt8 and

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transforming growth factor (TGF)-β superfamily members, do not function as community factors (Standley et al., 2001). The signaling pathways downstream of eFGF that mediate the community effect have not yet been identified, nor is it clear whether eFGF functions as an instructive or permissive signal in this instance.

A community effect is also evident in mouse myogenesis, and this requirement can be distinguished from the requirement for inductive signals provided by the neural tube and notochord (Cossu et al., 1995). Cells explanted from presomitic paraxial mesoderm and newly formed somites must be surrounded by a minimum of 30-40 similar cells in order to differentiate in response to axial tissue induction. The community effect observed in murine myogenesis differs from that seen in *Xenopus* in that fewer cells are required and the need for group contact occurs later. This latter distinction is likely to be related to differences between amphibians and mammals in the development of somites and myotomes (Cossu et al., 1995). The factor (or factors) that mediates the community effect in mammalian myogenesis is unknown; FGFs play complex roles in muscle development in higher vertebrates (discussed by Flanagan-Steet et al., 2000; Marics et al., 2002) and, by analogy with the *Xenopus* system, are appealing candidates.

Muscle precursor cells in intact embryos make adhesive contacts with their neighbors. The identification of a secreted, diffusible protein as a community factor raises the possibility that these simply keep cells within a community together to prevent diffusion and dilution of the factor. However, such interactions would be expected to influence cell morphology and behavior. In fact, early studies of the community effect in *Xenopus* myogenesis demonstrated that stable expression of the myogenic determination factor, MyoD, depends on N-cadherin-mediated cell-cell interactions (Holt et al., 1994). A relationship between cadherin-based cell adhesion and the production or actions of eFGF has not been identified, and the role of cadherins in this system could indeed be limited to providing the 'glue' for a community of cells. However, cadherin-mediated signal transduction (either direct or indirect) might also be involved, and the studies discussed below argue it is an important regulator of vertebrate myogenesis.

Specification and differentiation of cells in the myogenic lineage

During myogenesis, the changes in gene expression and cell morphology that accompany differentiation must be coordinated in a spatiotemporal fashion. This might occur through regulation of these processes by signaling initiated through cell-cell adhesion. The proteins involved could regulate many stages of myogenesis, including specification of uncommitted precursor cells through the community effect and/or other mechanisms, myoblast attraction, morphological changes that precede fusion (elongation and alignment) and fusion itself. Several classes of adhesion molecule and related proteins are implicated in cell-contact-based regulation of myogenic differentiation, in particular members of the cadherin family and Ig superfamily.

Cadherins

Classical cadherins play a central role in cell-cell adhesion (Wheelock and Johnson, 2003). Cadherin-mediated adhesion occurs through calcium-dependent, homophilic binding of the ectodomains of cadherin cis-dimers on the surface of apposing cells. The cytoplasmic tails of cadherins interact with proteins linked to the filamentous actin cytoskeleton, including β- and α-catenins, thus coupling ectodomain interactions to dynamic intracellular tensile forces (Wheelock and Johnson, 2003; Yap and Kovacs, 2003). In addition, cadherin-based adhesion can activate signal transduction pathways both directly (i.e. as a direct result of cadherin ligation) and indirectly through the ability of juxtacrine[†] ligands to contact their receptors as a consequence of cadherin-based adhesion (Yap and Kovacs, 2003).

Several classical cadherins have been implicated in regulation of myogenesis. N-cadherin is expressed throughout avian and murine myogenesis, from newly forming (i.e. first) somites through myoblast fusion and innervation of myofibers (Cifuentes-Diaz et al., 1994; Linask et al., 1998; Radice et al., 1997). A variety of in vitro approaches have documented its role in myogenic differentiation. Antibodies that neutralize N-cadherin inhibit differentiation of chick primitive streak epiblast cells (myogenesis being the preferred developmental pathway of such cells in vitro), primary chicken embryo myoblasts, and the C2 murine myoblast cell line (Charrasse et al., 2002; George-Weinstein et al., 1997; Knudsen et al., 1990b). BHK cells, which express MyoD but not cadherins and differentiate poorly, can be stimulated to express muscle markers by forced expression of cadherins and culture in three-dimensional aggregates (Redfield et al., 1997). In this system, expression of either N-cadherin or E-cadherin (the latter is not usually found in cells of the myogenic lineage) increases levels of sarcomeric myosin, indicating that cadherin-mediated adhesion, rather than N-cadherin-specific signaling, promotes differentiation (Redfield et al., 1997).

The use of recombinant cadherin ectodomains coupled to planar substrates or beads permits analysis of the downstream effects of cadherin ligation in the absence of cell-cell contact and consequent juxtacrine signaling. Such reagents induce lateral clustering of cadherins at sites of attachment, recruitment of catenins, and regulation of the actin cytoarchitecture (reviewed by Yap and Kovacs, 2003). In myoblasts at low cell densities, they induce differentiation similar to that observed in cells cultured on plastic substrata at high cell densities; by contrast, cells plated at low densities on fibronectin or poly-L-lysine differentiate much less efficiently (Gavard et al., 2004; Goichberg and Geiger, 1998). Cadherin ligation results in accumulation of the p21 and p27 cyclin-dependent kinase inhibitors and cell-cycle withdrawal, and increased expression of myogenin and muscle-specific sarcomeric proteins (Charrasse et al., 2002; Gavard et al., 2004; Goichberg and Geiger, 1998).

Thus, activation of cadherin signaling can largely substitute for cell-cell contact in differentiation of cultured myoblasts. Activation of N-cadherin in myoblasts by substrate-coupled ectodomains alters the activation state of Rho GTPases, stimulating RhoA and diminishing Rac1 and Cdc42 activities (Charrasse et al., 2002). Rho GTPases are established regulators of actin dynamics (Hall, 1998), and cadherin-

[†]Juxtacrine signaling occurs when a ligand presented from the plasma membrane of a cell binds to and activates a receptor on the surface of an apposing cell.

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induced control of their activities is likely to help drive the morphological changes that occur during differentiation. Furthermore, RhoA signals through serum response factor (SRF) to enhance muscle-specific transcription and promote myogenesis (Wei et al., 1998). Cadherin-mediated activation of RhoA might therefore be a mechanism by which changes in morphology and gene expression can be coupled during differentiation.

Despite the compelling evidence that supports a role for N-cadherin in myogenesis in vitro, genetic proof of such a role is still awaited. Mice lacking N-cadherin display small, irregularly shaped somites and die by embryonic day (E) 10 owing to defects in heart development, prior to the expression of markers of skeletal muscle differentiation (Radice et al., 1997). Explanted E9.0 somites from these animals cultured in the presence of neural tube do, however, express muscle-specific myosin heavy chain (Radice et al., 1997). Furthermore, myoblasts obtained from N-cadherin-heterozygous mice rendered genetically null in culture by selection for enhanced resistance to G418 differentiate normally [this selection strategy exploits the design of the N-cadherin targeting vector, which includes a functional *neo* gene (Charlton et al., 1997)]. These results indicate that N-cadherin is not essential for myogenesis; this is probably because additional classical cadherins compensate for its loss.

M-cadherin and R-cadherin are also expressed in myotomes and early muscle masses (Moore and Walsh, 1993; Rosenberg et al., 1997), and have been implicated as regulators of myogenesis. Peptides that are predicted to block homophilic M-cadherin interactions inhibit production of muscle-specific structural proteins and myotube formation by rat L6 myoblasts, as does antisense-mediated reduction of M-cadherin levels (Zeschnigk et al., 1995). Ectopic expression of R-cadherin in cultures of E-cadherin-null embryonic stem (ES) cells rescues the ability of such cells to aggregate and, strikingly, induces the formation of striated muscle in ES-cell-derived teratomas (Rosenberg et al., 1997). Interestingly, neither E-cadherin nor N-cadherin displays this latter property. This indicates that R-cadherin might play some non-redundant role in myogenesis (Larue et al., 1996). Nevertheless, mice lacking M-cadherin or R-cadherin are viable and do not display obvious defects in muscle development (Dahl et al., 2002; Hollnagel et al., 2002). This was particularly unexpected in the case of the M-cadherin-null animals because it is the only classical cadherin whose expression is primarily muscle specific (Hollnagel et al., 2002; Moore and Walsh, 1993).

Analyses of mice lacking cadherins shown to regulate myogenesis in vitro have thus been inconclusive. Whether loss of one of these cadherins results in deregulated expression of other cadherins is not yet known, but dramatic alterations in N-cadherin expression are not observed in skeletal muscle or cultured satellite cells derived from M-cadherin-null animals (Hollnagel et al., 2002). Analysis of tissue-specific and double or triple mutants may be required to clarify the compensatory relationships among these proteins and additional adhesion molecules. A conditional N-cadherin knockout has recently been constructed and therefore skeletal-muscle-specific deletion of this gene is now possible (Kostetskii et al., 2005). A recent study revealed that zebrafish N-cadherin (encoded by the *parachute* locus) and M-cadherin play non-redundant roles in migration and sorting of slow-twitch muscle cells during myotomal patterning (Cortes et al., 2003). Nevertheless, loss of either cadherin does not result in obvious changes in expression of myosin heavy chain in these cells, indicating that if classical cadherins are involved in myogenic specification or differentiation in zebrafish, redundancy occurs in this species as well. Given these results, despite the significant differences in somite structure between zebrafish and mice, it would be interesting to assess whether mouse cadherin mutants display alterations in fiber-type patterning.

NCAM

Cell-surface proteins of the Ig superfamily mediate cell-cell interactions during numerous developmental processes, functioning in morphogenesis and cell migration (Brummendorf and Lemmon, 2001). They possess ectodomains consisting of one or more Ig repeats, often followed by one or more fibronectin type III (FNIII) repeats. Most have a single membrane-spanning region and cytoplasmic tail; these tails can have catalytic functions or have no obvious resemblance to other known proteins. Many family members serve as homophilic and heterophilic cell adhesion molecules, whereas others lack adhesive functions and serve as receptors for secreted ligands (Brummendorf and Lemmon, 2001; Huber et al., 2003).

The neural cell adhesion molecule (NCAM), which harbors five Ig and two FNIII repeats in its ectodomain and participates in homophilic cell-cell adhesion, was the first Ig superfamily protein implicated in myogenesis. NCAM exists as multiple polypeptides that arise from alternative splicing and can include a muscle-specific domain (MSD) between the FNIII repeats that is a site of *O*-linked glycosylation (Lyons et al., 1992). In mice, *NCAM* mRNA is expressed in epithelial somites and throughout myogenesis; during this process, specific isoforms display dynamic changes in their expression levels (Lyons et al., 1992). Overexpression of specific isoforms of NCAM in C2 myoblasts enhances cell-cycle withdrawal, creatine kinase activity and the rate of myoblast fusion (Dickson et al., 1990; Peck and Walsh, 1993); furthermore, mice expressing an NCAM-encoding transgene display enhanced secondary myoblast fusion (Fazeli et al., 1996). Interestingly, *O*-linked glycosylation at the MSD positively regulates myoblast fusion in vitro, whereas polysialylation at specific asparagine residues of Ig repeat 5 inhibits fusion (Suzuki et al., 2003); polysialylation of NCAM is known to increase intermembrane repulsion and abrogate cell-cell adhesion (Johnson et al., 2005).

Multiple studies in which primary chick myoblasts were treated with antibodies to NCAM led to the conclusion that NCAM-mediated homophilic adhesion is involved in initial contacts between myoblasts in the early stages of fusion, but that cadherin-based adhesion is ultimately sufficient for myogenesis in vitro (Grumet et al., 1982; Knudsen et al., 1990a; Mege et al., 1992). Furthermore, reported studies of *NCAM*-null mice have not described significant defects in myogenesis (although the mice do have small, functionally impaired neuromuscular junctions), and primary myoblasts from such animals differentiate indistinguishably from wild-type myoblasts in vitro (Charlton et al., 2000; Cremer et al., 1994; Rafuse et al., 2000). Although a mammalian NCAM2 exists (Paoloni-Giacobino et al., 1997), there are no reports of

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its expression or function during myogenesis; whether this potential paralog and NCAM function redundantly is yet to be determined. However, the modest effects of NCAM loss-of-function in vivo and in vitro suggest that the enhanced myotube formation observed upon overexpression of NCAM occurs as a result of a general increase in cell-cell adhesion and, despite dynamic changes in the levels of its various isoforms during muscle development, that NCAM plays only a small role in myogenesis.

CDO, BOC and neogenin

CDO (also known as CDON) has five Ig and three FNIII repeats in its ectodomain and a long intracellular region that has no sequence resemblance to other proteins (Kang et al., 1997). It is expressed in first somites, dermomyotomes and myotomes, and in developing, but not adult, muscles (Kang et al., 1998; Mulieri et al., 2000). Overexpression of CDO in myoblast cell lines accelerates and enhances differentiation, promoting expression of muscle-specific proteins and myotube formation (Kang et al., 2003; Kang et al., 1998). A recombinant, secreted, soluble form of the CDO ectodomain has a dominant-negative effect, retarding differentiation (Kang et al., 1998), and RNA interference (RNAi) directed against CDO blocks differentiation (W.Z. and R.S.K., unpublished). Skeletal muscle development is delayed throughout the embryo in *Cdo*$^{-/-}$ mice (Cole et al., 2004). This developmental delay lasts until at least E14.5, but myogenesis ultimately recovers in *Cdo*$^{-/-}$ animals, which have properly organized, but somewhat smaller, muscle groups at birth (Cole et al., 2004). Therefore, CDO is not essential for skeletal muscle development, but is required for the process to occur in a timely and efficient fashion. This is reminiscent, at least superficially, of the delayed myogenesis seen in mice that lack either Myf5 or MyoD, which also recover from an early deficit (Braun et al., 1992; Kablar et al., 1997; Rudnicki et al., 1992). Primary myoblasts from *Cdo*$^{-/-}$ mice differentiate defectively in culture, expressing reduced levels of myogenin and forming very few myotubes (Cole et al., 2004). Therefore, unlike promyogenic cadherins, which may be compensated for by related proteins, CDO clearly plays a non-redundant role in muscle development.

Myogenic differentiation and muscle-specific gene expression depend on the myogenic bHLH transcription factors, which cooperate with the MEF2 family in a positive-feedback network (Molkentin and Olson, 1996; Yun and Wold, 1996). How this network is regulated by extracellular signals is under intensive study; the expression pattern, cell-surface location and promyogenic effects of CDO in vitro and in vivo have suggested that it might transduce such signals. CDO stimulates post-translational activation of myogenic bHLH factors, including MyoD, Myf5 and myogenin, in transient assays in cultured fibroblasts (Cole et al., 2004). It enhances their heterodimerization with E proteins (e.g. E12 and E47), probably by inducing hyperphosphorylation of the E proteins (Cole et al., 2004). The signaling pathways involved are as yet unidentified, but one site in E47 known to be targeted by ERK mitogen-activated protein (MAP) kinases is underphosphorylated in *Cdo*$^{-/-}$ myoblasts and so this pathway might play a part (Cole et al., 2004; Nie et al., 2003). Interestingly, the *Cdo* gene itself is induced in myoblasts and fibroblasts by MyoD and/or MyoD-induced transcription factors (Cole et al., 2004; Kang et al., 1998). Thus, CDO activates myogenic bHLH factors, which, in turn, enhance *Cdo* expression, as part of the positive-feedback network introduced above.

CDO appears to exert its promyogenic effects as part of a complex with other Ig superfamily proteins and cadherins, at sites of cell-cell contact. BOC is the protein most closely related to CDO, although the resemblance is restricted to their extracellular and transmembrane domains (Kang et al., 2002). *Cdo* and *Boc* have very similar expression patterns during mouse embryogenesis, and overexpression of BOC in myoblast cell lines results in the same type of accelerated differentiation seen with overexpression of CDO (Kang et al., 2002; Mulieri et al., 2002). CDO and BOC form cis complexes through direct association of their ectodomains and cytoplasmic tails. Interestingly, a recombinant, soluble form of the BOC ectodomain can promote myogenic differentiation in vitro, which indicates that the intracellular region is dispensable for BOC activity, at least in this assay. This contrasts with the effects of the soluble CDO ectodomain, and suggests that CDO and BOC exert their effects as components of a receptor, in which the role of BOC is primarily extracellular and that of CDO includes intracellular signaling. Consistent with this notion is the observation that the negative effects of soluble CDO predominate over the promyogenic effects of soluble BOC (Kang et al., 2002). Although these results suggest that CDO and BOC work together, no loss-of-function studies on BOC have been reported.

A second Ig superfamily protein that associates with CDO is neogenin (Kang et al., 2004). Neogenin is a receptor for the netrin family of secreted ligands, which play wide roles in morphogenesis, most notably axon guidance (Hinck, 2004; Huber et al., 2003). Netrin-1-mediated signaling through its receptor, DCC (a neogenin paralog), regulates morphological responses at the tips of migrating growth cones and stimulates NFAT-dependent transcription (Graef et al., 2003; Huber et al., 2003). Specific members of the NFAT family of transcription factors regulate various stages of myogenesis, including myoblast fusion (Horsley and Pavlath, 2002). Thus, netrin-initiated signal transduction is another possible mechanism by which morphological changes and transcription might be coordinated during myogenesis. Cultured primary myoblasts and myoblast cell lines express neogenin and netrin-3 proteins, and the genes encoding neogenin, DCC, netrin-1 and netrin-3 are variously expressed in somites and developing muscle during mouse embryogenesis (Gad et al., 1997; Keino-Masu et al., 1996; Puschel, 1999; Wang et al., 1999). Overexpression of neogenin in C2C12 cells promotes myotube formation, whereas knocking down neogenin inhibits this (Kang et al., 2004). Similarly, treatment of these cells with exogenous netrin enhances formation of myotubes.

Neogenin increases myogenic bHLH- and NFAT-dependent transcription in reporter assays, and netrin treatment stimulates production of the activated form of NFATc3; however, the increase in myotube formation produced by these factors is not accompanied by widespread increases in expression of muscle-specific proteins (Kang et al., 2004). Neogenin binds to CDO (and, at least in transient assays, to BOC) in a cis fashion and, interestingly, *Cdo*$^{-/-}$ myoblasts cannot activate NFATc3 in response to netrin, despite producing wild-type levels of

neogenin (Kang et al., 2004). CDO may therefore be part of a complex of proteins that includes the primary netrin receptor, neogenin, and together transduce netrin-initiated signals in myoblasts. However, the distinct effects of CDO and neogenin in C2C12 cells indicate that not all the effects of CDO are exerted in this fashion. Although mice lacking neogenin display incompletely penetrant perinatal lethality (Srinivasan et al., 2003), muscle development in these animals has not been analyzed. It will be interesting to assess myogenesis in these animals and in mice lacking both CDO and neogenin.

CDO and BOC (or complexes of CDO and BOC) do not bind to each other in a trans fashion, nor do they seem to mediate cell-cell adhesion (Kang et al., 1997; Kang et al., 2002; Kang et al., 1998). However, when ectopically expressed in COS cells, they colocalize at sites of cell-cell contact (J.S.K. and R.S.K., unpublished). This suggests that CDO and BOC might be drawn to such sites by interaction with bona fide cell-cell adhesion molecules. In fact, CDO and BOC each bind to N-cadherin and M-cadherin in a cis manner that does not require cadherin-mediated adhesion (Kang et al., 2003). These complexes contain β-catenin and α-catenin and are enriched at sites of cell-cell contact between myoblasts (Kang et al., 2003) (J.S.K. and R.S.K., unpublished observations for α-catenin). Neogenin is also associated with cadherins in myoblasts (Kang et al., 2004). Stable expression in C2C12 cells of a CDO mutant that cannot associate efficiently with cadherins interferes with muscle-specific gene expression and myotube formation. This suggests that interaction with cadherins is required for CDO to stimulate myogenesis (Kang et al., 2003). Because CDO and BOC interact with both N-cadherin and M-cadherin, some of the apparent genetic redundancy among cadherins in myogenesis might be explained by a common ability to associate with these Ig superfamily members. However, whereas CDO seems to depend on interactions with cadherins, it is not clear that the converse is true; that is, whether cadherins require association with CDO or other Ig superfamily proteins to exert their promyogenic effects.

We can envisage the following model for the function of a cell-surface complex of cadherins and Ig superfamily proteins in skeletal myogenesis (Fig. 1). Multiple signals emanate from this complex. CDO signals induce hyperphosphorylation of E proteins, resulting in enhanced formation of heterodimers of myogenic bHLH factors with E proteins, and increased muscle-specific transcription. Activation of CDO might involve its functioning, with BOC, as a receptor for an unidentified ligand. Netrin-3/neogenin signaling activates myogenic bHLH factor- and NFATc3-dependent transcription and is a good candidate for one mechanism that regulates the morphology of myoblasts, perhaps through pathways implicated in myogenesis that are activated by netrin-1 and DCC in neurons [e.g. those involving MAP kinases, calcineurin and Rho GTPases (Campbell and Holt, 2003; Forcet et al., 2002; Graef et al., 2003; Huber et al., 2003)]. The cadherins mediate cell-cell adhesion by tethering to the actin cytoskeleton and also activate RhoA, which in turn can alter both cytoskeletal dynamics and SRF-mediated transcription. Note that many ligands stimulate clustering of their cognate receptors to initiate signaling, and CDO and BOC might be clustered by interacting with cadherins and/or neogenin, which have their own specific ligands (cadherins on apposing cells and netrins, respectively). The need to invoke a ligand specific for CDO and BOC is therefore not strictly necessary, because they could function as cofactors within this complex.

Although the proteins pictured in Fig. 1 can be co-immunoprecipitated from cultured myoblasts, it is not known how many distinct complexes exist, or whether the composition of such complexes changes during myogenesis. Similarly, the

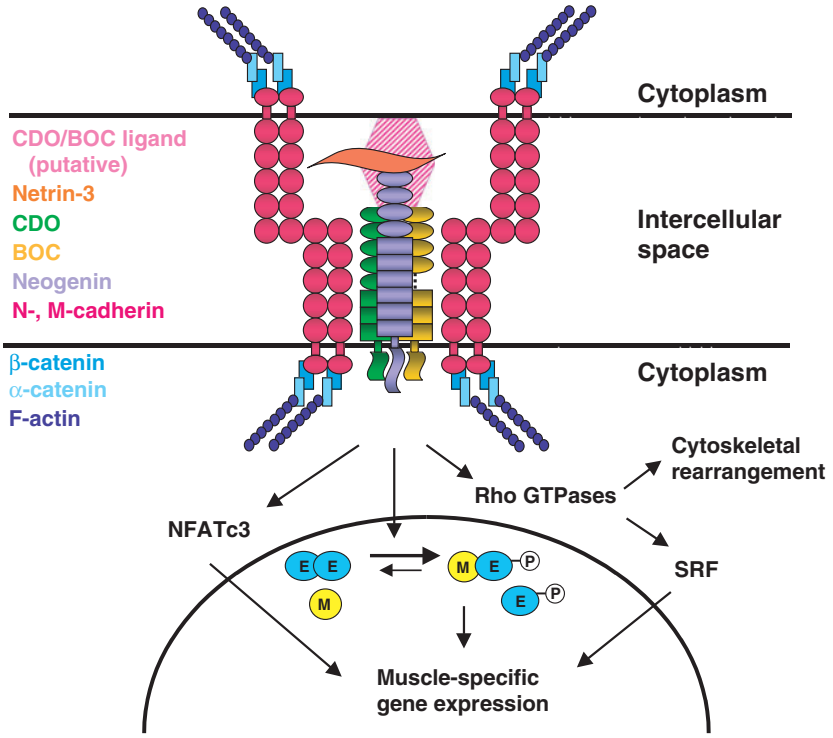

Fig. 1. Model for a promyogenic cell-surface complex. A complex of CDO, BOC, neogenin and its ligand netrin-3, and N- and M-cadherins and the cadherin-associated proteins β-catenin and α-catenin is found at sites of myoblast cell-cell contact. A CDO/BOC ligand, whose uncertain existence is indicated by stripes, is also shown. Multiple signaling pathways emanate from this complex, resulting in activation of transcription factors that drive muscle-specific gene expression, including: heterodimers of myogenic bHLH factors (M) and E proteins (E), SRF and NFATc3; regulation of the actin cytoskeleton; and ultimately differentiation into multinucleated myofibers with functional sarcomeres. Note that the figure as drawn is not meant to imply any particular stoichiometry or complex formation between CDO, BOC and neogenin, or between these proteins and cadherins. Note also that, although CDO, BOC and neogenin are shown on one cell and their ligands on the other, all these proteins are produced by myoblasts, and the signals shown here may occur in a bidirectional fashion. Finally, signals generated by these adhesion molecules and receptors are expected to cooperate with signals derived from soluble factors and the extracellular matrix during myogenesis. See text for further information.

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fraction of the total amount of each factor present in complexes, the stoichiometry of binding, and any mutually exclusive interactions remain to be determined. Nevertheless, the data strongly support the notion that the components of the complex are interdependent in terms of some (though probably not all) of their myogenic activities [e.g. CDO depends on cadherins for its function, and BOC and netrin depend on CDO for theirs (netrin presumably through neogenin) (Kang et al., 2003; Kang et al., 2002; Kang et al., 2004)]. Interactions with cadherins might bring CDO and BOC to sites of adhesive cell contact so that they, in turn, can interact with additional factors at these sites that relay signals downstream. Because CDO and BOC do not have clearly demonstrable adhesive activity, their association with cadherins provides a mechanism for their localization to sites of cell-cell adhesion. Recruitment of neogenin to these complexes might occur in a similar fashion through interaction with CDO and/or BOC or perhaps with cadherins themselves. Assembly of a multiprotein complex at sites of cell-cell contact that contains multiple signaling and adhesive components makes biological sense, because such complexes could permit diverse signaling events that regulate morphological and transcriptional responses to be coordinated during differentiation, from a site in the cell that must undergo dramatic changes as myoblasts elongate, align and ultimately fuse. Furthermore, because CDO activates MyoD, and MyoD, in turn, enhances *Cdo* expression, the myogenic positive-feedback network includes components that reside on the cell surface that can be directly responsive to extracellular cues.

Conclusions and perspectives

Recent work has provided the beginnings of a molecular understanding of such phenomena as the community effect and additional promyogenic effects of cell-cell contact. Loss-of-function phenotypes in genetically tractable model organisms would be expected to shed light on the specific steps in myogenesis that are regulated by individual factors implicated as mediators of cell-contact-dependent events. However, the apparent compensatory or redundant roles of several such factors tested have, thus far, made firm conclusions difficult. Much of the evidence for contact-mediated regulation of myogenesis therefore comes from studies performed in vitro, most often on the differentiation of determined myoblasts in culture. It will be important to provide further genetic proof for the factors implicated; the generation of double mutants of specific cadherins will be particularly interesting in this regard. Identification of the signaling pathways that directly mediate the effects of cell-cell contact, the mechanisms by which cell contact activates these pathways, and how these pathways ultimately interface with cytoskeletal proteins and transcription factors to regulate the dramatic changes that occur during differentiation are topics of high interest and current research. It will be even more challenging to integrate the knowledge generated by such studies with information about how myogenesis is regulated by soluble factors and extracellular matrix proteins, and their respective receptors, which also play key roles in muscle development (Gullberg et al., 1998; Pirskanen et al., 2000; Pownall et al., 2002; Puri and Sartorelli, 2000; Schwander et al., 2003). Skeletal myogenesis has long provided a paradigm for understanding problems in lineage specification, cell differentiation and tissue-specific gene expression, and we expect that this will continue as work over the next several years begins to illuminate the relationships between the myriad extracellular signals involved in these processes.

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