

A Conserved Developmental Role of Adhesion Molecules in Cardiac and Skeletal Myogenesis

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Abstract

Cell adhesion molecules are associated with the commitment of precardiac cells to the myocardial lineage. They are also important in the formation of the somitic structure, and subsequently, skeletal muscle development in the myotome. Specifically, the N-cadherin/6-catenin complex appears to have an important role in the events leading to myogenesis in both types of muscle. The immunostaining patterns of N-cadherin/B-catenin in the above regions are dynamic and developmentally regulated. They are associated with the early stages of commitment and differentiation of the myogenic populations. The known roles of fibronectin, NCAM and N-cadherin are reviewed in both cardiac and skeletal myogenesis. It appears that cell adhesion-mediated events have been evolutionarily conserved and underlie important mechanisms leading to myogenic compartments and early stages of myofibrillogenesis in the invertebrate and vertebrate embryos.

Key words: N-cadherin, fibronectin, N-CAM, heart, skeletal, muscle, myogenesis.

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Local signals in the different regions of the embryo will determine the pathway of differentiation of cells. Thus, a seemingly homogeneous population of cells in the heart-forming mesoderm may have the same potential fate, for example, to become a cardiac cell, but eventually can follow one of the alternative pathways that are available for its specific region, eg. to become an endocardial endothelial cell or a myocardial cell. In many cases, the choice may be influenced by signaling molecules originating outside of the group of cells in question. This is termed induction. Alternatively, the signal may arise from specific cells within the population that are competent to respond to the signal. These cells will begin to differentiate along a different pathway. This is termed lateral specification. This may be followed by cell sorting and the formation of new compartments in that embryonic region. As a result, a field is cut up into finer and finer regions of specialized cells. A key component of signaling events leading to a new compartment involves modulation of cell adhesion molecules.

Adhesive interactions appear necessary to permit specialized cells to remain together and also may regulate the differential gene expression that causes cells to become specialized. An understanding of cell differentiation predicates a need to understand how

molecular pathways leading to differentiation are coordinated with expression of adhesion molecules that are critical for organizing differentiating cells into functional tissue compartments within the embryo.

Expression of three superfamilies of adhesion molecules are coordinated to form specialized tissues. These include cell-substratum adhesion molecules, as characterized by the fibronectin-integrin type of cell-extracellular matrix interaction; calcium independent cell-cell adhesion molecules typified by the immunoglobulin-like superfamily of which N-CAM is a member; and the calcium dependent cell-cell adhesion superfamily, the cadherins. Adhesive interactions mediated by all three classes of adhesion molecules can lead to cell signaling and alterations in gene expression. In addition, these adhesion systems may communicate with each other and together modulate cell responses in a coordinate fashion.

At present little is known about coordination of adhesion-mediated signaling pathways relating to muscle development. This review is limited to a discussion of the patterns and possible role(s) of cell adhesion molecules in the early events of cardiac and skeletal muscle leading to phenotypic differentiation. For a discussion of the regulatory genes associated with somitic and cardiac differentiation other published

reviews are suggested (eg. for somites see [7], for heart related see [11, 41, 59]).

It is becoming apparent that there are a number of distinctive similarities in adhesion-mediated processes leading to cardiac cell differentiation and to myotome cell commitment and myoblast differentiation in the somite. The commonalities suggest that the adhesion-related mechanisms of muscle development have been conserved in both cardiac and skeletal muscle, whereas the transcription factors involved in the two myogenic pathways have diverged [41].

Cardiac Cell Commitment and Differentiation

Fibronectin

In the stage 4+5 chick embryo, fibronectin (FN) first appears symmetrically within the bilateral heart forming regions at the mesoderm-endoderm interface [35]. Reflecting an anterior/posterior progression of embryonic development in general, fibronectin expression spreads from its initial expression in anterior bilateral regions more posteriorly across the heart field and down the length of the embryo. As a result of this anterior/posterior progression of synthesis, a gradient of FN becomes apparent between stages 7-8 in the chick heart-forming areas. It was shown that this gradient is also involved in the directional precardiac cell migration toward the lateral walls of the developing foregut [34, 36, 37]. During this period of increased fibronectin deposition in the underlying matrix, the cardiomyocyte population undergoes epithelialization, with the basal surfaces of the cells displaying an enrichment of the integrin receptor, as the cells become associated with the FN fibrillar meshwork in their substratum.

Perturbation of fibronectin results in abnormal heart development [13, 27, 36]. It is apparent that normal heart development is perturbed in fibronectin-antibody treated chick embryos in a rostrocaudal manner often resulting in cardiabifida. Alternatively, wide hearts develop at the midline. The variability of tubular heart formation most likely is dependent upon the time of exposure to the antibody. Abnormalities in heart development, as well as in the development of the vascular network, are reported in a fibronectin-null transgenic mouse model [13]. Heart tissue can develop, but has abnormal organization. Severity of defects in the FN-deficient embryos apparently relates to the background strain of mouse used to produce the transgenics [11].

There are differences in fibronectin expression and localization in the mouse and chick embryos during cardiogenesis. The differences relate to the steepness of the gradient of differentiation (unpublished observations). In the chick, a tubular heart is first formed which begins to beat at approximately 33 hr of

development. Mouse heart differentiation, including myogenesis, displays a much steeper anterior-posterior gradient of cardiac development. For example, anteriorly, as soon as the anterior cardiomyocytes differentiate, the cells begin to beat. Posteriorly within the heart-forming region, the cells may just be showing changes associated with the first steps of differentiation and appear as a homogenous mesoderm population. For this reason it should not be assumed that all processes among different vertebrates will be identical as to the timing of expression of specific molecules or events during histogenesis and organogenesis. Molecules involved in signaling and differentiation may be similar, but the timing of their expression and patterning may vary. It is by examining the differences and similarities among the vertebrates that much essential information may be gained in relation to the function of particular molecules during development (see also [1]).

N-cadherin/β-catenin complex

N-cadherin is a member of a large superfamily of calcium-dependent cell adhesion molecules that have a demonstrably important role during morphogenesis, especially in association with cell sorting events [53, 54]. The interaction of N-cadherin with intracellular proteins is necessary for its adhesive function. The intracellular α -, β - and γ -catenins form a complex with cadherins. β -catenin binds to the cytoplasmic domain of the transmembrane cadherins [57]. β -catenin is the conserved vertebrate homologue of the armadillo protein that plays an important role in *Drosophila* embryo segmentation [39] and in embryonic boundary development (in *Xenopus* [12], in the chick see [29]). It is becoming apparent that N-cadherin in association with β -catenin plays an equally important role in boundary specification and subsequent differentiation of the cardiac compartment [30, 32].

In the early chick embryo at stage 4, N-cadherin/ β -catenin are localized ubiquitously throughout the mesoderm. At stage 5 there appears to be a signaling event in the most anterior regions of the bilateral heart-forming areas whereby N-cadherin/ β -catenin become restricted to central areas in patch-like clusters in an anterior to posterior progression. Apposing cell membranes within a cluster show a high intensity of N-cadherin/ β -catenin localization. These N-cadherin positive regions display a periodicity [30]. Often large, apparently mitotic cells are associated with these clusters. It is within these regions that the first small cavities appear that marks the beginning of the pericardial coelom. These small cavities enlarge as a result of Na/K-ATPase activity [31], and coalesce with neighboring cavities to eventual form bilateral coeloms that will subsequently coalesce to form the pericardial coelom.

Adhesion and myogenesis

N-cadherin/ β -catenin expression demarcates where the ventral, splanchnic, cardiac compartment separates from the dorsal, somatic mesoderm. Before separation N-cadherin/ β -catenin complex becomes restricted to cell surfaces primarily on the same dorsal-ventral plane, appearing almost as a "line" between the initial expressing clusters. It then becomes localized to the apical-lateral sides of cells lining the ventral splanchnic side of the developing coelom where tight junctions are forming [32] (Fig. 1). These cells epithelialize, elongate, and associate with the underlying dense matrix of FN that is present on their basal side. Concomitantly, the first phenotypic characteristics associated with cardiomyocytes appear, i.e. electrical activity [24] and cardiac myo-fibrillogenesis which is initially apparent at the apical side of the cells [19, 55, 56].

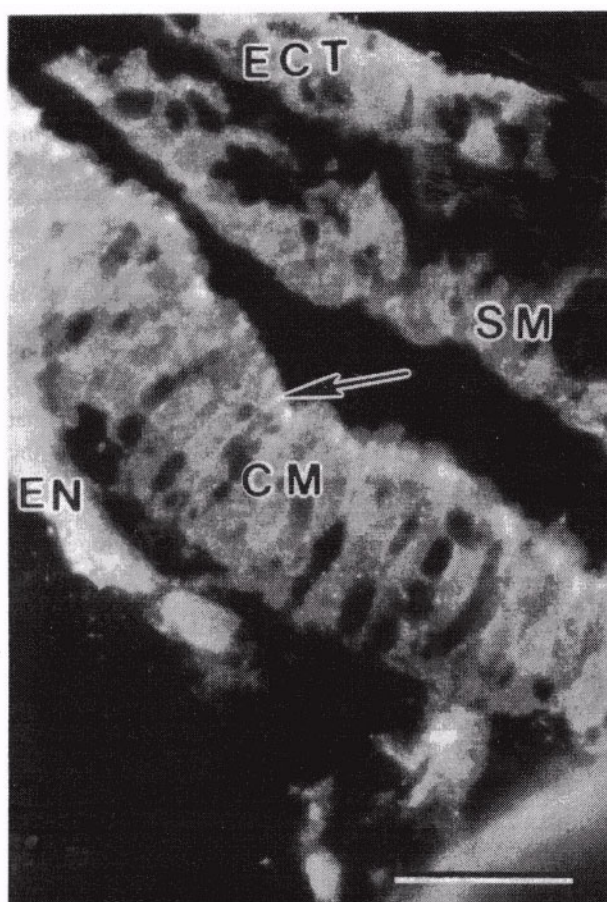


Figure 1. N-cadherin is expressed on apical-lateral surfaces after epithelialization of splanchnic cardiac mesoderm cells in the anterior bilateral chick heart-forming regions (see arrow). N-cadherin remains stably expressed by the myocardium throughout development. ECT, ectoderm; CM, cardiac mesoderm; SM, somatic mesoderm and EN, endoderm. (From Linask, 1992. Used with permission). Scale bar = 25 μ m.

N-cadherin perturbation in the chick using the perturbing antibody NCD-2, or by genetic manipulation in a transgenic N-cadherin null mouse embryo, results in severely abnormal heart development and cardiac myogenesis [32, 48]. In the avian model, perturbation of N-cadherin function results in an arrest of cardiac cell epithelialization and myofibrillogenesis in a temporal fashion [32]. Significantly, after stage 8, the rostrocaudal progressive signaling across this region is complete and antibody treatment no longer has an affect in these embryos. In younger embryos where anterior signaling has been completed, the apparently committed, epithelialized anterior cells go on to differentiate regardless of the presence of the antibody, while the mesenchymal posterior cells remain sensitive to perturbation. Thus, there appears a narrow developmental window relating to epithelialization during which N-cadherin function is necessary for normal cardiomyocyte differentiation. In the N-cadherin null mouse model, however, a loose association of cardiac cells can form a thin myocardium that is only weakly contractile. Also the expression of β -catenin in these null embryos appears to be relatively weak at the cell membrane compared to normal, control animals. At a time in development when diffusional forces can no longer supply nutritional demands and heart function becomes essential, the N-cadherin null embryos die.

N-cadherin appears to be involved in myofibrillogenesis [15, 22, 51]. If one removes chick precardiic mesoderm explants at stage 5 when the cells appear as a homogeneous population of mesenchymal cells, and incubate them for up to 20 hours, the normal progression of nascent myofibrillogenesis can be observed in a spatiotemporal fashion by immunostaining for α -actinin, N-cadherin, and α - or β -catenin (See Fig. 2A-D) (also [22]). If N-cadherin is perturbed with the NCD-2 perturbing antibody, cardiac myofibrillogenesis is arrested and myofibrils are disassembled [22]. This arrest of myofibrillogenesis is also seen *in vivo* with antibody perturbation [32]. Further evidence that N-cadherin expression is necessary for the organization of myofibrils is that endocardial endothelial cells contain myofibrils possibly at a time when they initially express N-cadherin [cf. 33, 55]. Later, when N-cadherin is down-regulated and vascular cadherin is apparently up-regulated during endothelial cell differentiation, myofibrils are disassembled. In the N-cadherin-null mouse model a similar effect of loss of N-cadherin adhesion on cardiac myofibrillogenesis is evident. The myocardium is thin and only weakly contractile [48]. That some myofibrillogenesis occurs suggests the presence of another cadherin which is able to partially compensate for N-cadherin, but not completely.

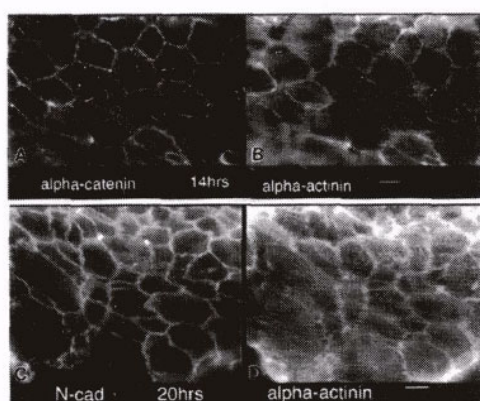


Figure 2. Precardiac mesoderm explants from stage 5 chick embryos were removed and incubated for various time periods. After 14 hrs in culture, explants were double-immunostained for a-catenin and a-actinin (2A and 2B, respectively), a-catenin localizes to cell membranes and a-actinin is beginning to show organization into microfibrils extending to the membrane areas. By 20 hrs incubation in a double stained preparation, N-cadherin continues to be expressed at cell boundaries (Fig. 2C; as does a- and fi-catenin, not shown), a-actinin now shows incorporation into sarcomeric structures (Fig. 2D). (Photographs courtesy of Dr. Kyoko Imanada-Yoshida, Mie University, Japan). Scale bar - 10 μ m.

In later stages N-cadherin remains essential for normal heart development. As the myocardial wall of the vertebrate heart changes from a simple epithelium to a trabeculated structure, N-cadherin is up-regulated [42]. N-cadherin appears to play a role in the homotypic interactions between nonepithelial migratory myocytes during trabeculation of the embryonic heart. Thus, in addition to N-cadherin's known roles in myocyte epithelialization and intercalated disc formation, N-cadherin appears to be part of other morphoregulatory processes in association with the development of the myocardium.

N-CAM

N-CAM a member of the calcium-independent, immunoglobulin-like superfamily of adhesion molecules and is present also during the above-described early stages of heart development, as well as later [5]. During early stages NCAM is expressed by the myocardial population, as well as the endocardial [32]. The patterning of N-CAM expression, however, does not change during compartmentalization as the cardiac cells epithelialize, as was observed for N-cadherin expression. N-CAM is ubiquitous in its expression

throughout the bilateral mesoderm and remains uniformly expressed into the tubular heart stages. In immunohistochemical studies of hearts from stage 18 embryos, isoforms of N-CAM containing the muscle-specific domain colocalized with Z-discs [6]. *In vitro* studies indicate that this localization does not depend on cell-cell contact and may involve instead cell-extracellular matrix interactions. While the N-cadherin knockout is an embryonic lethal, the N-CAM knockout mouse can go through birth and is viable. Hence, N-CAM has an adhesive function during heart development, but apparently of lesser importance than N-cadherin.

Somitogenesis

Somites form in an anterior to posterior manner as paired structures arising from the mesenchymal segmental plate located on either side of the developing neural tube in the vertebrate embryo. Each somite appears when a group of homogeneous appearing cells within the most rostral end of the segmental plate undergoes a change in cell organization from mesenchyme to an epithelialized rosette. The cells display cell-cell interactions associated with a true epithelium. Later the epitheliod structure begins to show changes associated with the diversification of the somite. Cells in the ventromedial portion of the somite become the sclerotome which gives rise to the vertebral column. The dorsolateral cells become the dermomyotome which later subdivides to give rise to the dermis of the trunk on the dorsal side. The cells that remain sandwiched between the dermatome and the sclerotome forms the myotome. It is the myotome that will eventually give rise to the axial skeletal muscle. Associated with morphoregulation of somitogenesis, specific modulation of cell adhesion molecules is observed.

Fibronectin

The pattern of fibronectin distribution can be correlated with the initiation of somitogenesis in the anterior portion of the segmental plate [43]. Fibronectin (FN) localizes predominantly to the dorsal and ventral lateral aspects of the segmental plate. This corresponds to the region where it is delineated from the lateral mesoderm. A sparse punctate pattern of FN is commonly seen in the body of the segmental plate. As the somite separates from the segmental plate, FN appears at its medial surface. In the nascent somite, FN is distributed in association with cells on the periphery. As the somite undergoes diversification to form sclerotome and denamyotome, FN distribution becomes discontinuous and is most prominent in the migrating sclerotome. The same temporal and topological changes of fibronectin are seen in mouse somitogenesis as in the chick embryo (compare [43,

44]). A notable exception is that during somite epithelialization in the mouse, the cells do not form a closed vesicle as it does in the chick and the mesial portion of the forming somite does not become epithelial before the migration of sclerotome cells [44].

During early stages of somitogenesis, cells in the anterior portion of the segmental plate undergo compaction as they show increased cell-cell adhesion. The aggregation of segmental plate cells *in vitro* is stimulated by fibronectin [28]. This stimulation of aggregation and compaction of segmental plate cells by FN can be seen in isolated segmental plate cells, in isolated segmental plates, and in intact embryos removed from yolk. Thus, it appears that the developmental window during which FN is necessary for somitogenesis relates to its acting as a "trigger" to signal the process of compaction [27]. Treating segmental plate cells in culture with the fibronectin-derived synthetic peptide GRGDS, which is recognized by the integrin receptor, increases isolated segmental plate cell-cell adhesion, but not cell to substratum adhesion. Epithelialization of the cells accompanies compaction as the cells form a complete somite. Unpublished observations showing that soluble RGD-peptides stimulate N-cadherin synthesis during somitogenesis (Yamada and Lash, personal communication) suggest complementary function arising from an interaction between cell-cell and cell-matrix adhesion molecules. Such cross-talk between members of the different families of adhesion molecules has been shown also for neural crest cells [40]. Thus, as in cardiomyocyte development and epithelialization, the basal surfaces of both somitic and cardiac cells are closely associated with fibronectin and apical surfaces with N-cadherin. Somites do not form in fibronectin null embryos [13]. It can be deduced from fibronectin antibody perturbation experiments in the chick and from genetic manipulation of fibronectin in a transgenic mouse model that fibronectin is an important molecule both for normal heart development and for somitogenesis. Whether FN is important for myotome compartmentalization remains to be determined because the null embryos do not live long enough for somite diversification to occur.

Cadherin/β-catenin complex

As the cell-substratum adhesion molecule fibronectin undergoes changes in its patterning in the somitic regions of the embryo, N-cadherin/6-catenin also undergoes specific changes in its expression during early somitogenesis. A role for N-cadherin in somitogenesis is based upon experiments that demonstrate modulation of N-cadherin localization during somitogenesis [9, 20, 54]. We have observed similar changes in N-cadherin expression and have extended these studies to analyze the details of β-

catenin expression (an important note for N-cadherin/catenin adhesion in morphoregulation of early somitogenesis is now in press. See Linask et al.: *Dev Biol* 1998; 201: in press). In the segmental plate there is an ubiquitous localization of N-cadherin and β-catenin. Just before the new somite forms in the anterior most region of the segmental plate, a small number of cells begin to cluster and form small foci of cells that express higher levels of N-cadherin/6-catenin. This is a likely indication of the initial clustering of more adhesive cells. Eventually the clusters enlarge, as more cells are drawn into the clusters. In the more anterior somitic regions several such clusters may be seen. It is within this narrow developmental time-period that one can produce anomalous or supernumerary somites by perturbing with N-cadherin antibody (Linask et al., 1998) or heat shock [47]. Each N-cadherin/β-catenin expressing cluster can give rise to a somitic structure (Linask et al., 1998). These clusters eventually coalesce into one large somitic cluster, as the cells elongate and epithelialize. N-cadherin/β-catenin becomes apically localized, while FN remains expressed at the basal surface. Concomitantly, the somite cells undergo compaction. The cytoskeleton of the somitic cells is associated via bridging molecules at its two poles, apical and basal, with two different signaling adhesion systems. This raises the possibility that cross-talk occurs between these two adhesion systems to coordinate morphogenetic events. The same molecules and processes are evident during cardiac cell compartmentalization.

Following epithelialization, the somite undergoes diversification into the dermamyotome and sclerotome populations. N-cadherin and α- and β-catenins remain associated with the myotome that will differentiate into skeletal muscle cells (Fig. 3). N-cadherin is down-regulated in the sclerotome that will form the vertebrae. Recent *in vitro* studies suggest that N-cadherin mediated cell-cell adhesion is involved both in the commitment of skeletal muscle precursors and their terminal differentiation [14, 49]. Cells with the potential to form skeletal muscle are present in the chick embryo epiblast prior to gastrulation [14]. If epiblast cells are treated with N-cadherin perturbing antibody *in vitro*, the percentage of MyoD and myosin expressing cells is greatly reduced. Inhibition of N-cadherin mediated adhesions also blocks the differentiation of cultured somite and segmental plate cells [14]. If BHK fibroblast-like cells are transfected with N-cadherin, 6-catenin is upregulated and strong cell-cell adhesion is induced [49]. When BHK cells were cultured as three-dimensional aggregates, N-cadherin expression enhanced withdrawal from the cell cycle and stimulated differentiation into skeletal muscle, as measured by increased sarcomeric myosin expression. A

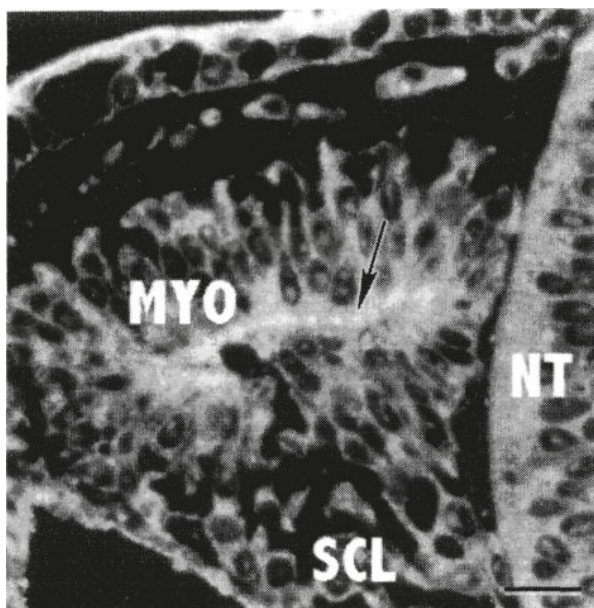


Figure 3. β -catenin localizes (arrow) to the apical-lateral membranes of the myotome population of cells in the somite. Sclerotome cells are seen migrating toward the neural tube-notochord area. MYO, myotome; SCL, sclerotome; NT, neural tube. Scale bar = 10 μ m.

nonfunctional mutant of N-cadherin failed to promote skeletal muscle differentiation in BHK cells. This suggests an adhesion-competent cadherin is required for muscle differentiation [49].

The diversification of cells in the heart region follows a similar pattern in its modulation of adhesion molecules, as occurs in the somite. In the bilateral heart regions it is the myocyte population that retains N-cadherin expression and uses this molecule for the site of initiation of myofibrillogenesis [22, 29, 30, 32, 33]. N-cadherin is down-regulated in the endocardial endothelial cells [33]. Similarly, in the somite, it is the myotome population that gives rise to muscle cells that maintains N-cadherin expression at its cell-cell junctions. Thus, both cardiac and skeletal myogenesis appear to depend upon N-cadherin-mediated cell to cell associations that occur between a specific group. This is called the community effect [8, 16-18, 21].

In addition to N-cadherin several other cadherins, such as R-cadherin and M-cadherin, are present in somites and are differentially regulated during somite diversification [23]. M-cadherin (muscle-cadherin) in addition to N-cadherin is expressed in mammalian skeletal myoblasts [46]. *In vivo* M-cadherin mRNA is detected exclusively in skeletal muscle. It is not known whether these cadherins have different functions than that of N-cadherin during skeletal myogenesis or, if other cadherins are present in the early stages of myocardial differentiation.

N-CAM

A spatiotemporal analysis of N-CAM expression during somitogenesis indicates that this cell-cell adhesion molecule is also involved in myogenesis. Segmental plate cells are weakly stained for N-CAM. During compaction, N-CAM is significantly increased among the aggregating cells. In the nascent somite, the whole surface of epithelialized cells stain for N-CAM, while N-cadherin is localized apically. This pattern for these two adhesion molecules is similar to that observed during cardiomyogenesis. During diversification of the somite into dermamyotome and sclerotome, N-CAM remains expressed in both populations. The adhesion of isolated segmental plates are only weakly affected by N-CAM antibody, while those incubated in the presence of N-cadherin perturbing antibody are dissociated [9]. Furthermore, antibodies to N-cadherin, but not to N-CAM inhibit myogenesis in cultures of somite and segmental plate cells [14]. Therefore, as in heart development, N-cadherin appears to have a major role in somitogenesis, while N-CAM appears involved to a much lesser degree [10, 32].

During later stages of skeletal muscle development, N-CAM has been suggested to have a role in myoblast fusion [26]. In the mouse it has been shown that as muscle differentiation proceeds, several N-CAM isoforms are expressed [38, 58]. In the chick the 155 kD form NCAM was expressed specifically and transiently during embryonic day 11 to day 14 in chick muscle. This coincides with the period of extensive myotube formation and this isoform of N-CAM may have an important role in this process.

Community effect

The term "community effect" has been used to describe the phenomenon observed in development when cell to cell interactions are necessary for the activation and completion of the differentiation of a specific cell type [18]. This effect is applicable to muscle development. It is not until the dorsal boundary of anterior bilateral, splanchnic mesoderm is defined by N-cadherin/ β -catenin in the heart fields, that a compartment of cells joined by N-cadherin junctions is set up. This concomitantly leads to epithelialization of this compartment followed by the first signs of phenotypic cardiac cell differentiation that appears to be activated by this compartmentalization event. In a similar manner during skeletal myogenesis in the *Xenopus laevis* embryo a group of cells of 50 to 200 cells was necessary to get muscle gene activation when normal muscle progenitor cells were transplanted to a different region of a whole embryo [25]. Cells transplanted singly changed fate. Subsequently, it was shown that N-cadherin is involved in activation of myogenic genes and the muscle differentiation pathway in *Xenopus*, chicken and mouse cells [14, 21, 49]. In

addition injections of dominant negative N-cadherin mRNA into cells of *Xenopus* embryos suppress *MyoD* expression in muscle progenitor cells [21]. Similarly, in the N-cadherin null transgenic embryo, cardiac muscle differentiation and somitogenesis is severely compromised [48]. A cardiac compartment is present, but normal myofibrillogenesis does not occur *in vivo*. Also normal somites do not form. If somites from the N-cadherin null embryos are placed in culture, myogenesis does occur; however, these cells do express another cadherin. Thus, cadherin mediated interactions appear to play an important role in community signaling during cardiac and skeletal muscle differentiation.

Myogenic regulatory pathways and cadherin-mediated cell adhesion

In amphibian, avian and mammalian embryos, skeletal myogenic cells express members of the Myo-D family of helix-loop-helix transcription factors. The precardiac cell, however, does not utilize the same myogenic regulatory genes that are present in skeletal muscle determination. Regulatory genes associated with skeletal muscle myogenesis as *MyoD*, *myf 5*, *myogenin*, and *MRF4* are not expressed in cardiac muscle. Rather during cardiac cell commitment genes as *Nkx2.5* and *GATAs 4,5, and 6* are expressed (for review see [41]). Genes, however, that are activated downstream, as *MEF2C* are found in both skeletal and cardiac muscle types. Most cells in the cardiogenic region and in the newly formed somite appear to be initially multipotent and become committed to specific fates in response to instructive signals from surrounding cell types (see [50, 59]). It appears that gradients of signaling molecules may result in boundary specification and the activation of specific patterns of genes. How these signaling molecules determine boundaries and regulate myogenesis in the somite and the heart is unknown, but it appears that concentrations of signaling factors are important, as myogenesis occurs in only a narrowly defined subset of cells, both in the cardiogenic field, as well as in the somite. For example, activation of *pax 1* and *QmyoD* during sclerotome and myotome specification appears to occur through distinct signal/response mechanisms [2]. This study indicates that *Shh* may be an important signaling molecule for both *QmyoD* and *pax1* and that a gradient of signaling molecules along the anterior-posterior axis of the embryo is present and a differential response of somitic cells occurs to various concentration of these molecules.

A number of regulatory genes are known to be involved in the regulation of somite formation. *Paraxis* is a basic helix-loop-helix (bHLH) transcription factor that is expressed in the paraxial mesoderm immediately prior to somite formation and in newly formed epithelial somites. As somites mature, *paraxis* expression becomes localized to the epithelial cells of the

dermomyotome [3, 52]. Mice homozygous for a *paraxis* null mutation were unable to form epithelioid somitic structures, but rather the paraxial mesoderm appeared to be segmented into loose mesenchymal units of approximately the correct size and periodicity of somites [4]. Later the paraxial mesoderm did not show normal segmentation of the somites. Interestingly, the null mutants in terms of skeletal muscle development displayed differentiated muscle cells, but the patterning of muscle was abnormal. It is not known whether expression of cell adhesion molecules as N-cadherin is affected in the mutants, but the observation that epithelialization does not occur suggests that some aspect of the N-cadherin-B-catenin pathway is affected. It also indicates that possibly the most critical aspect of epithelialization and formation of tight adherens junctions is to maintain the correct cell-cell associations in a three-dimensional manner for correct patterning and differentiation of tissues to occur in the embryo. Each cell within a compartment finds itself within concentration gradients of dorsal/ventral, anterior/posterior and left/right signaling factors. Essentially cell adhesion fixes the cells in a three dimensional manner which then allows patterning of cells into tissues to be defined. Adhesion molecules in turn can feedback to act in a signaling capacity [45].

Boundaries created by signaling molecules appear to be stabilized by adhesion molecules to form defined cellular compartments. In both cardiac and skeletal muscle populations N-cadherin plays a necessary role in the formation of the myogenic compartment. N-cadherin mediated adhesion accompanying cell epithelialization may be a common, evolutionarily conserved, event that triggers or activates phenotypic cardiac, as well as, skeletal myogenesis. N-cadherin in this context can be considered a competence modifier that defines the compartment or subset of cells within which subsequent differentiative events of myogenesis can be coordinated.

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