Neural Cell Adhesion Molecule (NCAM) and Myoblast Fusion

C. A. Charlton, W. A. Mohler,[1] and H. M. Blau[2]

Department of Molecular Pharmacology, Stanford University School of Medicine,
Stanford, California 94305

Considerable evidence points to an involvement of neural cell adhesion molecule (NCAM) in myoblast fusion. Changes in the level of NCAM expression, isoform specificity, and localization in muscle cells and tissues correspond to key morphogenetic events during muscle differentiation and repair. Furthermore, anti-NCAM antibodies have been shown by others to reduce the rate of myoblast fusion, whereas overexpression of NCAM cDNAs increases the rate of myoblast fusion compared to controls. In this study we have used a novel fusion assay based on intracistronic complementation of lacZ, in combination with fluorescent X-gal histochemistry and immunocytochemistry to assess levels of NCAM expression in individual muscle cells. Our results indicate that a substantial proportion of newly fused myoblasts have NCAM expression levels unchanged from the levels of the surrounding unfused population suggesting that increased expression of NCAM is not required for wild-type myoblasts to fuse. Moreover, pure populations of primary myoblasts isolated from mice homozygous null for NCAM and therefore lacking the molecule, when placed in differentiation medium, consistently fused to form contractile myotubes with kinetics equivalent to wild-type primary myoblasts. We conclude that the increase in expression of NCAM, although typically observed during myogenesis, is not essential to myoblast fusion to form myotubes. © 2000 Academic Press

Key Words: cell adhesion; fusion; muscle differentiation; NCAM.

INTRODUCTION

Neural cell adhesion molecule (NCAM) is among the most prevalent of cell adhesion molecules found in vertebrates. It is expressed in a wide range of early embryonic tissues at sites of induction, but later in development is found predominantly in nervous tissue and in developing muscle where it is thought to function in such processes as axonal bundling, migration of neurons, myogenesis, muscle innervation, and synaptogenesis (Balak *et al.,* 1987; Baldwin *et al.,* 1996; Covault *et al.,* 1986; Edelman, 1985; Levi *et al.,* 1987; Thiery *et al.,* 1982; Walsh and Doherty, 1997). NCAM exists as three major polypeptides with more than 20 different isoforms, all generated from a single 70-kb gene (Cunningham *et al.,* 1987; Dickson *et al.,* 1987; Doherty and Walsh, 1991; Goridis and Brunet, 1992; Moore *et al.,* 1987; Rutishauser and Landmesser, 1991). Expression of

NCAM isoforms occurs in a highly tissue-specific and developmentally regulated manner, suggesting that the different NCAM isoforms have distinct functions.

In muscle development, both the level of expression of specific NCAM isoforms and their location within the cell have been shown to change in a manner that suggests that NCAM-mediated adhesion may play a role in timing or directing key events of myogenesis, such as the myoblast fusion that accompanies muscle formation or regeneration following injury (Covault and Sanes, 1986; Covault *et al.,* 1986; Daniloff *et al.,* 1986; Fredette *et al.,* 1993; Moore *et al.,* 1987; Tosney *et al.,* 1986). Numerous reports have implicated NCAM in myoblast adhesion and fusion (McDonald *et al.,* 1995). NCAM expression markedly increases in populations of differentiating myoblasts and the fusion-competent myotubes resulting from myoblast fusion (Covault *et al.,* 1986; Moore *et al.,* 1987). Increased adhesion between myoblasts accompanies their ability to fuse (Knudsen and Horwitz, 1977). Fab fragments of antiserum against NCAM significantly inhibit adhesion and have also been shown to reduce the rate of primary chick myoblast fusion (Knudsen *et al.,* 1990). Similarly, mouse C2C12 myoblasts

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[1] Current address: Laboratory of Molecular Biology, University of Wisconsin, 1525 Linden Drive, Madison WI, 53706. Fax: (608) 262-4570. E-mail: wamohler@facstaff.wisc.edu.

[2] To whom correspondence should be addressed. Fax: (650) 725-2952. E-mail: hblau@cmgm.stanford.edu.

overexpressing human NCAM cDNAs exhibit increased rates of fusion compared to controls, and a species-specific monoclonal antibody to human NCAM eliminates this increase, strongly suggesting that the rate of fusion is directly influenced by the additional NCAM expressed (Dickson *et al.,* 1990; Peck and Walsh, 1993). In addition to an overall increase in the level of NCAM expression during myogenesis, an increase in specific isoforms of NCAM is correlated with fusion; a 140-kDa transmembrane isoform predominates in proliferating myoblasts and is progressively replaced during fusion by a 125-kDa NCAM isoform (Covault *et al.,* 1986; Mège *et al.,* 1992; Moore *et al.,* 1987). Removal of the 125-kDa NCAM isoform from the surface of chick myoblasts inhibits adhesion and fusion while overexpression in transgenic mice enhances fusion (Fazeli *et al.,* 1996; Knudsen *et al.,* 1989).

Some reports, however, minimize the role of NCAM in myoblast fusion. Cifuentes-Diaz *et al.* (1993) and Mège *et al.* (1992) observed only slight inhibition of fusion in the presence of anti-NCAM antibodies. Furthermore, reports of the targeted mutation of the single mouse gene encoding the different NCAM isoforms have not described gross defects in muscular development or alterations in the viability of mutant animals (Cremer *et al.,* 1994; Moscoso *et al.,* 1998; Wood *et al.,* 1998). These results have been interpreted to suggest either that NCAM is not essential to myogenic fusion or that redundant proteins can substitute for its function *in vivo.*

This paper describes two types of studies designed to rigorously test the hypothesis that NCAM expression is essential to myoblast fusion. Both employ variations of a sensitive β-galactosidase complementation assay developed in our laboratory (Mohler and Blau, 1996; Charlton *et al.,* 1997). In these assays, myoblasts were infected with retroviruses encoding either the $\Delta\mu$ or the $\Delta\omega$ mutant β-galactosidase (β-gal) peptide and cocultured under growth or differentiation conditions. The first study used β-gal complementation visualized by a novel, highly sensitive fluorescent X-gal substrate in conjunction with immunohistochemical staining of NCAM to monitor at the level of an *individual* cell whether an increase in NCAM expression correlates with the onset of fusion competence. Previous studies were only able to address fusion and NCAM expression levels in *populations* of fusing myoblasts. The second type of study used β-gal complementation in a sensitive, quantitative biochemical assay of fusion in cultures of primary myoblasts derived from NCAM-null mice compared to cultures derived from wild-type mice. Since myoblasts in culture are not under selective pressure to fuse, detection of subtle differences in timing or efficiency of myoblast fusion would be possible by this method. Our results show clearly that although NCAM may be involved in muscle formation, its role in myoblast fusion is not essential.

MATERIALS AND METHODS

Culture of Cells

C2F3 myoblasts, nontransformed, highly fusogenic derivatives of C2C12 myoblasts (Rastinejad and Blau, 1993), were grown in C2F3 growth medium consisting of 15% calf serum (CS) and 5% fetal bovine serum (FBS) (HyClone) in Dulbecco's modified Eagle's medium (DMEM) (Irvine Scientific).

Two breeding pairs of homozygous NCAM-null mice (Cremer *et al.,* 1994) were provided by J. Merlie of Washington University. Primary myoblasts were dissociated as previously described (Rando and Blau, 1994) from muscle tissue of three neonatal (approximately 1 week of age) littermates, the progeny of a mating of homozygous NCAM-null adult mice. Cells were subsequently passaged in primary myoblast growth medium consisting of 40% Ham's F10/40% DMEM/20% FBS plus 2.5 ng/ml bFGF (Promega) on dishes coated with Type I collagen (Sigma; concentration 0.1% in 0.1 N acetic acid overnight and then rinsed with sterile water). The NCAM-null primary myoblasts isolated from neonates were pooled for subsequent culture. Wild-type myoblasts were similarly isolated from three neonatal C3H mice, pooled, and cultured on collagen.

Infection of Populations of Myoblasts with Complementing Mutants of β-Gal

Two populations of each myoblast type, C2F3, C3H wild-type, or NCAM-null, were separately transduced with retroviral constructs encoding nonfunctional complementing β-gal mutants, either $\Delta\mu$ or $\Delta\omega$ as previously described (Mohler and Blau, 1996). When these mutant peptides are expressed together in the same cell or come together through cell fusion, they are able to form a functional enzyme that can be visualized or quantified using different substrates.

Fluorescent Histochemistry

Glass coverslips (No. 1 thickness) were sterilized by baking and then coated with Type I collagen (Sigma; concentration 0.1% in 0.1 N acetic acid) at 4°C overnight. Coverslips were rinsed with sterile water and placed in 35-mm culture wells.

C2F3 myoblasts, expressing individually either $\Delta\mu$ or $\Delta\omega$ mutants of β-gal, were cultured separately under normal growth conditions. At time zero, trypsinized cells were suspended either in differentiation medium (DM) consisting of 5% horse serum (HS) (Hyclone) in DMEM or in C2F3 growth medium, mixed in equal proportion ($\Delta\mu$ cells and $\Delta\omega$ cells), and plated at a density of 10^6 per well (5×10^5 cells of each complementing cell type) on collagen-coated cover-slips. After a time in coculture, cells were fixed for 5 min at room temperature with freshly prepared 4% paraformaldehyde (Polysciences) in PBS, rinsed twice and stored at 4°C in PBS until staining was performed.

All steps of the three-layer immunolabeling procedure were performed at 4°C. Fixed cells were incubated in blocking buffer (PBS + 10% HS) for 30 min. Antibodies were diluted in blocking buffer. Each antibody incubation proceeded for 2 h. The rat monoclonal antibody MAB310 (Chemicon) against mouse NCAM was diluted 1:1000. A biotinylated goat anti-rat antibody (Vector) was diluted 1:250. Cy5-labeled streptavidin (Amersham) was diluted 1:1000. Four 10-min washes were performed with 2 ml of blocking buffer between labeling steps. After the final Cy5–streptavidin

incubation, cells were rinsed twice with blocking buffer and twice with PBS.

Fluorescent X-gal staining solution contained 25 μg/ml 5-bromo-6-chloro-3-indolyl-β-D-galactopyranoside (Fluka) and 100 μg/ml Fast Red Violet LB (Sigma) and was filtered (0.45 μm) before being added to cells. β-Gal was allowed to activate the substrate at 37°C for 60 min. Each coverslip was then individually rinsed in 25 ml PBS at room temperature with gentle rocking for 1 h. Finally, coverslips and cells were mounted on glass slides in PBS and sealed with nail polish.

Slides were analyzed using the Molecular Dynamics Multiprobe 2010 confocal microscope located at the Stanford University Cell Sciences Imaging Facility. Fluorescent X-gal product was excited with the 488-nm line of the Kr/Ar laser and detected using a 515- to 545-nm bandpass FITC emission filter. Cy5 was excited with the 647-nm laser line and detected with a 660-nm long-pass emission filter. A 200-μm pinhole was used to maximize the depth of field. For scoring of cells, laser power and photomultiplier voltages were set empirically to allow faint detection of background for each fluorochrome. Fields were scanned simultaneously at both wavelengths, and both images were displayed side by side in pseudo-color, with the scale selected to show significant brightness above background. Cells were scored manually based upon the pseudo-color scale as positive or negative for β-gal and high or low for expression of NCAM. At least 50 fields were scanned for each slide analyzed. For pictorial display, β-gal activity is shown in green and NCAM immunocytochemistry is in red.

Western Immunodetection of NCAM in Wild-Type and NCAM-Null Muscle Cell Cultures

Protein was extracted from C3H wild-type or NCAM-null primary myoblasts grown either in growth medium for three days or in DM for 3 or 5 days. Equal amounts of protein, determined by Bradford assay, were run in lanes of an SDS–polyacrylamide gel and transferred to PVDF membrane (Millipore). Immunodetection was performed using rat monoclonal antibody against mouse NCAM (MAB310, Chemicon; diluted 1:1000), an antibody that recognizes all NCAM isoforms, followed by HRP-conjugated goat anti-rat antibody (Sigma; diluted 1:5000) and ECL detection (Amersham). Prior to harvesting, cultures were photographed using a Sony CCD IRIS/RGB color video camera attached to an inverted microscope.

β-Galactosidase Complementation Assay for Fusion of Primary Myoblasts

Populations of NCAM-lacking ($-/-$) and C3H wild-type ($+/+$) myoblasts were separately transduced with retroviral constructs encoding either $\triangle\omega$ or $\triangle\mu$, nonfunctional complementing mutant peptides of the β-gal enzyme (Mohler and Blau, 1996). Cells expressing one peptide were mixed at equal densities with cells of the same type expressing the complementing mutant peptide in DM, plated at 1.5×10^4 total cells/well of 96-well microplates. DM was replaced daily. At the indicated time points, cells were lysed and processed as described (Mohler and Blau, 1996) for chemiluminescent assay using the Galacton-Plus chemiluminescent detection kit (Tropix). Functional β-gal enzyme activity was measured using a MicroBeta 1450 luminometer (Wallac). Data are expressed as the means of eight replicate wells $\pm$ SD. Myoblast fusion is profoundly affected by cell density. Therefore, it was extremely important to plate equivalent numbers of cells and to analyze them

in the same experiment in order to compare cell types or culture conditions using this assay.

RESULTS AND DISCUSSION

NCAM Expression at the Level of Individual Fusing Myoblasts

Application of the β-gal complementation system to the study of fusion in nascent myotubes has several advantages. First, the signal produced by complemented β-gal activity provides a clear and rapid means of identifying the few small multinucleate cells that arise during the brief time span of the experiment and distinguish them from a vast population of unfused myoblasts. Furthermore, as the signal is fusion dependent, it assures that any binucleate cells being considered have arisen through fusion and not through incomplete cytokinesis, a phenomenon often seen even among nonfusing cell types in tissue culture. Second, mixing of complementing myoblasts at the outset of the experiment results in a defined starting point for analysis of the time course of fusion events. As β-gal-positive cells can only arise after the plating of a coculture (Mohler and Blau, 1996), multinucleate cells which may have formed spontaneously in the separate cultures before time zero do not produce active β-gal. Therefore, experiments can be focused entirely on those cells which have fused within a well defined time period. Third, a fluorogenic stain for β-gal allows simultaneous visualization of other molecules by immunofluorescence microscopy. This permits analysis of single cells in terms of two or more parameters, employing the sensitivity and quantitative tools available for epifluorescence microscopy. In these respects, the technique should be especially useful in studying properties of other fusogenic cell types such as trophoblasts, macrophages, and myoblasts in a range of species as well as the dynamics of cellular components, such as the cytoskeleton and various organelles, within newly formed syncytia.

In our study, the sensitive assay of complementation of β-gal was used as an indicator of recently fused cells using either a myoblast cell line, C2F3, or primary myoblasts isolated directly from mice and purified in culture to eliminate nonmyogenic cell types. First, the expression of surface NCAM was correlated with the onset of fusion competence at the level of the single cell. Complementing C2F3 myoblasts were mixed and plated in coculture at experimental time zero. Only β-gal-positive cells were scored after fixation and staining. Therefore, any fusion events that were analyzed had to have occurred between time zero and the time of fixation. Nascent β-gal-positive myotubes appeared in complementing cocultures after 22 and 34 h from the time of plating (Table 1), but at both times they constituted a very small percentage of the total number of cells per field of view (Fig. 1). Among these, β-gal-positive cells that displayed surface levels of NCAM that were similar to the majority of surrounding unfused myoblasts, and significantly lower than the NCAM levels

TABLE 1

Analysis at the Single Cell Level of NCAM Surface Expression in Newly Forming Myotubes

Culture	β-Gal-positive high-NCAM	β-Gal-positive low-NCAM	% Low-NCAM/total β-gal-positive
Differentiation medium (low serum)			
22 h	113	5	4.2
22 h	10	0	0.0
34 h	66	7	9.6
34 h	42	10	19.2
Total	231	22	9.5
Growth medium (high serum)			
22 h	58	12	17.1
34 h	14	6	30.0
34 h	9	9	50.0
Total	81	27	33.0
Low or high serum			
Total	317	51	13.9

Note. C2F3 myoblasts, expressing either the $\Delta\mu$ or $\Delta\omega$ mutant of β-galactosidase were cocultured in either high-serum growth medium (5% FBS/15% CS) or low-serum differentiation medium (5% HS) for 22 or 34 h, fixed, and stained for β-galactosidase complementation (β-gal-positive), a measure of cell fusion and for level of NCAM expression at the surface of the newly forming myotube. Each score shown is for an individual coverslip culture.

seen on the cells expressing the highest levels in the culture, were not exceptional, but instead were frequently observed (Table 1), indicating that high-level NCAM expression was not a prerequisite for fusion.

We tested the hypothesis that growth factor concentration might alter the requirement for NCAM in myoblast fusion. This was possible because C2F3 cells plated at high density are capable of fusing even in high-serum growth medium. β-Gal-positive/low-NCAM cells were found in cocultures plated in either low-serum differentiation medium or in high-serum growth medium (Table 1). While the overall frequency of β-gal-positive myotubes was lower in high-serum growth medium than in low-serum differentiation medium, the relative number of β-gal-positive fused cells that expressed a low level of surface NCAM was much higher in growth medium (33%) than in differentiation medium (9.5%). This result is consistent with NCAM expression increasing upon differentiation of myoblasts; however, it does not support the hypothesis that high NCAM expression is necessary for fusion to occur. Indeed, the value of 33% probably underestimates the actual number of low-NCAM myoblasts that have undergone fusion; a binucleate cell, for example, formed by the fusion of one low-NCAM myoblast and one high-NCAM myoblast is likely to display a relatively high level of NCAM. Table 1 shows the numbers of β-gal-positive/low-NCAM and β-gal-

positive/high-NCAM cells found in representative cultures under these two sets of culture conditions. Figure 1 shows examples of such β-gal-positive/low-NCAM cells that contrast clearly with both β-gal-positive/high-NCAM and β-gal-negative/high-NCAM cells in the surrounding culture. The results of these studies of NCAM expression levels in single newly fused myotubes strongly suggest that the increase of surface NCAM expression which occurs in differentiating skeletal muscle cells is a process that is concomitant with but not a prerequisite for fusion competence.

Our conclusion from analysis at the single cell level that fusion does not depend on high levels of NCAM expression assumes that an individual myotube, once fused, does not rapidly reduce its level of surface NCAM. Such a decrease would have to occur in less than 22 h between the fusion event (some time after experimental time zero) and the earliest time examined. A pulsatile NCAM expression pattern at the single cell level, although never described for myoblasts, is not inconsistent with the overall increase in NCAM expression which has been reported during myogenesis (Moore *et al.,* 1987). Furthermore, muscle fibers *in vivo* undergo loss and recovery of high NCAM expression after innervation and denervation, respectively (Covault *et al.,* 1986; Hahn and Covault, 1992), illustrating that NCAM can be regulated even after myogenesis is complete. However, the time scale of these changes is much longer than that delimited by the design of the experiment reported here. Additionally, immunofluorescence microscopy of cultures maintained for 5 days in differentiation medium showed most myoblasts had fused at this time but maintained NCAM expression at a high level on the cell surface (data not shown). This finding argues against the possibility that a rapid decrease in NCAM expression occurs after myoblasts fuse.

Analysis of Fusion in Wild-Type and NCAM-Null Myoblasts in Vitro

Targeted mutation of the NCAM gene in mice produced no gross defects in skeletal muscle (Cremer *et al.,* 1994); however, compensatory expression of another adhesion molecule could have occurred. *In vitro* there is no selective pressure to express NCAM since fusion is not essential to survival of cells in culture. In addition, more subtle changes in the time course or efficiency of fusion might become apparent in purified populations of primary NCAM-null myoblasts. We isolated primary myoblasts from a neonatal litter of offspring of breeding pairs of mice homozygous null for a targeted deletion of the gene encoding all forms of NCAM. Expression of NCAM in these cells was assessed by Western immunoblotting (Fig. 2A). As expected, levels of NCAM expression increased in cultures of wild-type myoblasts kept 3 and 5 days in differentiation medium. Wild-type myoblasts not exposed to differentiation medium, but kept 3 days in growth medium, had only a faint NCAM-reactive band of a higher molecular weight, while differen-
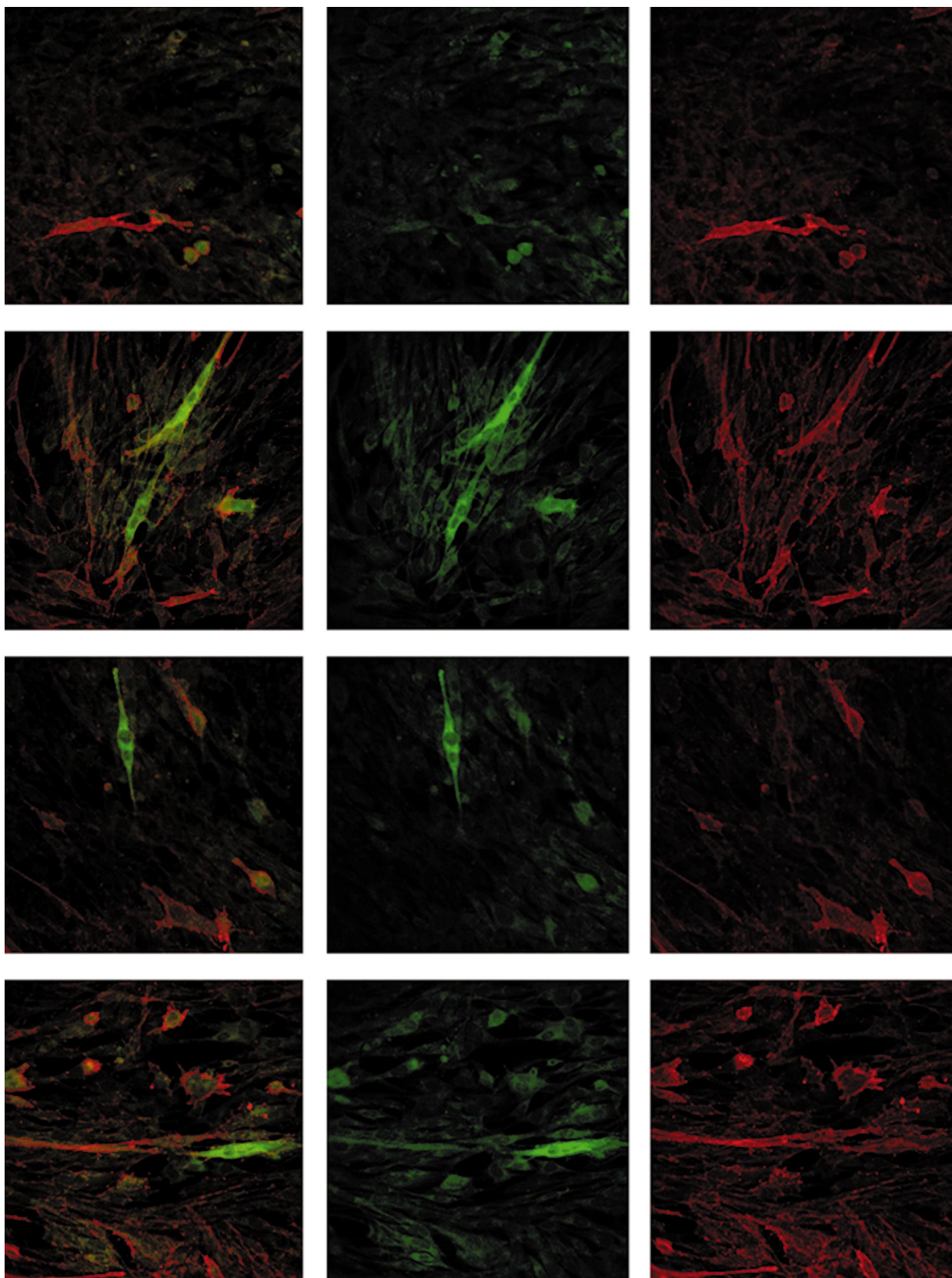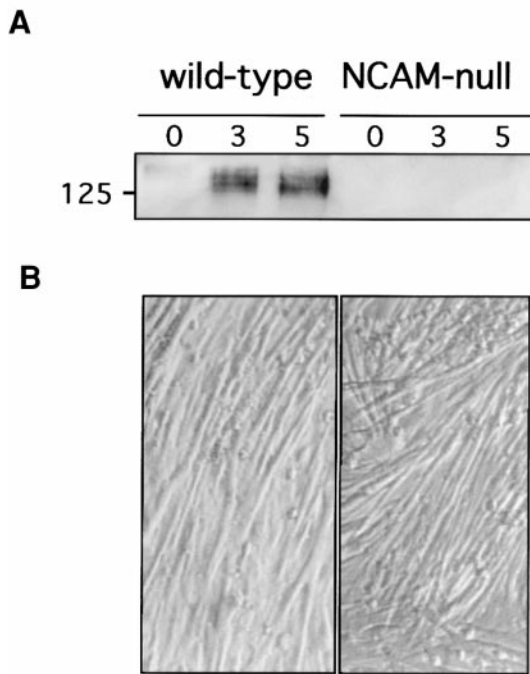
FIG. 2. Western analysis of NCAM expression in wild-type and NCAM-null myoblasts and myotubes. (A) Cells isolated from mice wild-type (lanes 1–3) or homozygous null (lanes 4–6) for the NCAM gene were grown for 0 days (lanes 1 and 4), 3 days (lanes 2 and 5), or 5 days (lanes 3 and 6) in low-serum differentiation medium. Amount of protein extracted from resulting cultures was equalized by the Bradford assay and analyzed by Western immunoblotting with anti-NCAM antibody that recognizes all isoforms of NCAM. (B) Five day cultures of wild-type (left) or NCAM-null (right) differentiated and contracting myotubes were photographed prior to harvesting for Western analysis (magnification, 140×).

FIG. 3. Kinetics of fusion in wild-type and NCAM-null myoblasts *in vitro*. A chemiluminescent assay was used to measure fusion of wild-type (+/+) or NCAM homozygous null (−/−) myoblasts. Separate populations of each cell type were transduced with retroviral vectors containing either the Δμ or Δω nonfunctional mutant of β-galactosidase. When cultured together for various times in DM, fusion of myoblasts was measured as functional β-galactosidase activity in chemiluminescent assay. Each point is the average of eight replicates ± SD.

tiated myotubes showed progressively more of the lower molecular weight NCAM isoform characteristic of myotubes. In cultures of NCAM-null myoblasts or fully differentiated fused and contracting NCAM-null myotubes, no expression of any NCAM isoform was detected (Figs. 2A and 2B). Primary myoblasts from NCAM-null mice fused with kinetics and efficiency equivalent to myoblasts of wild-type cultures (Fig. 3). These observations, together with the studies described above showing that fusion competence is independen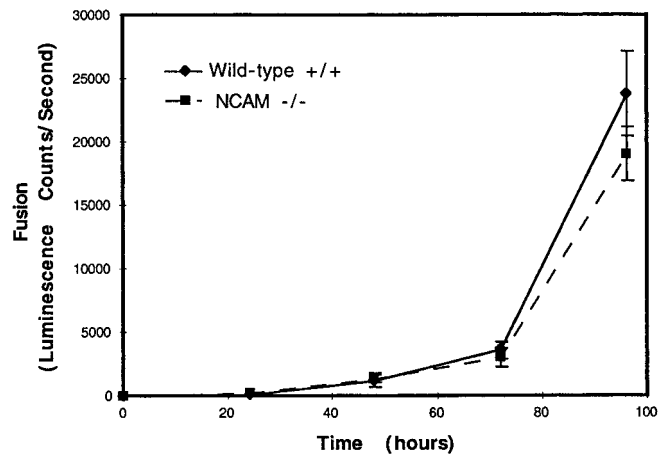t of increased NCAM expression at the single cell level, demonstrate clearly that NCAM is not essential for myoblast fusion to occur.

Two questions therefore arise. First, why in myogenesis should there be an increase in NCAM expression which is conserved across species? Second, what is the significance of studies in which overexpression of NCAM enhances the rate of fusion in cultured myoblasts and antibodies to NCAM decrease that rate? It is thought that NCAM may be important both in the higher order organization of muscle tissue and in the formation of neuromuscular contacts leading to synaptogenesis. In development, a high level of NCAM is transiently expressed in embryonic muscle and then lost in normal adult muscle except at neuromuscular junctions. The expression of a highly polysialylated form of NCAM has been temporally and ultrastructurally correlated with the separation of chick secondary muscle fibers from the primary fibers along which they form (Fredette *et al.,* 1993; Allan and Greer, 1998), and in neuromuscular development, the overall pattern of NCAM expression on muscle fibers *in vivo* parallels their time course of innerva-

FIG. 1. Correlation of β-gal activity with expression of NCAM at the single cell level. All panels in each horizontal row of the figure show the same field of cells. The left-most column is an overlay of images in the middle and right columns that show fluorescent X-gal staining for β-gal activity in green (middle column) and anti-NCAM immunostaining in red (right column). In horizontal row 1, there is one myotube with high NCAM staining that is β-gal negative. In row 2 there is one β-gal-positive/low-NCAM cell, two β-gal-positive/high-NCAM cells, and several β-gal-negative/high-NCAM cells. In row 3 there is one β-gal-positive/low-NCAM cell and several β-gal-negative/high NCAM cells. In row 4 there is one β-gal-positive/low-NCAM cell and several β-gal-negative/high-NCAM cells (magnification, 200×).

tion (Rieger *et al.,* 1985; Sanes *et al.,* 1986). Anti-NCAM antibodies have been reported to block the formation of contacts between neuron processes and myotubes *in vitro* (Rutishauser *et al.,* 1983) and have been reported to block reinnervation of synaptic sites in frog muscle basal lamina after surgical denervation (Mège *et al.,* 1990). Polysialylated NCAM has also been implicated in the branching and bundling patterns of axon outgrowth and in the innervation of developing and regenerating chick muscle (Landmesser *et al.,* 1988, 1990). In agreement with this putative role in muscle fiber innervation, NCAM, especially polysialylated NCAM expression, is also increased on young regenerating fibers of mice (Dubois *et al.,* 1994). However, in NCAM-null mice, although endplates of neuromuscular junctions were reduced, no deficits in motor function or ability to reinnervate were reported suggesting that effects upon these developmental processes must be minor or compensated for *in vivo* by other molecules. Thus, despite the abundance of reports to the contrary, we conclude that an essential function for NCAM in any aspect of neuromuscular development has yet to be established.

The question that remains is whether NCAM has a role intrinsic to muscle development independent of innervation. Several studies suggest that it does. Overexpression of NCAM in cultured myoblasts has been shown to enhance the rate of cell fusion in two studies (Dickson *et al.,* 1990; Peck and Walsh, 1993). An alternative explanation of these results is that increased overall adhesiveness can increase the rate of myoblast fusion. The finding that increased NCAM expression leads to the increased adhesiveness between myoblasts that accompanies the onset of fusion competence supports this finding (Knudsen and Horwitz, 1977). It should be noted that neither overexpression study includes controls which would allow a conclusion that adhesion mediated specifically by NCAM is important for the effect. Thus, it is quite possible that any other homophilic adhesion molecule, even one not normally expressed by myoblasts, could enhance myoblast fusion when expressed at high levels. Taken together, the lack of a consistent correlation at the single cell level between increased NCAM expression and the acquisition of fusion competence, the apparently normal development and growth of muscle in the NCAM-null mouse, and the ability of primary myoblasts from these NCAM homozygous null mice to differentiate and fuse in culture in a manner equivalent to wild-type myoblasts suggest that NCAM per se is not a required component of adhesion leading to myogenic fusion.

ACKNOWLEDGMENTS

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