The atypical Rac activator Dock180 (Dock1) regulates myoblast fusion *in vivo*

Mélanie Laurin*†, Nadine Fradet*, Anne Blangy‡, Alan Hall§, Kristiina Vuori¶‖, and Jean-François Côté*†‖**

*Institut de Recherches Cliniques de Montréal, Montréal, QC, Canada H2W 1R7; †Département de Médecine, Université de Montréal, Montréal, QC, Canada H3C 3J7; ‡Centre de Recherche en Biochimie Macromoléculaire, Centre National de la Recherche Scientifique, 34293 Montpellier, France; §Memorial Sloan Kettering Cancer Center, New York, NY 10065; ¶Burnham Institute for Medical Research, La Jolla, CA 92037; and **Division of Experimental Medicine, McGill University, Montréal, QC, Canada H3A 1A3

Edited by H. Robert Horvitz, Massachusetts Institute of Technology, Cambridge, MA, and approved August 4, 2008 (received for review June 10, 2008)

Dock1 (also known as Dock180) is a prototypical member of a new family of atypical Rho GTPase activators. Genetic studies in *Drosophila* and *Caenorhabditis elegans* have demonstrated that Dock1 orthologues in these organisms have a crucial role in activating Rac GTPase signaling. We generated mutant alleles of the closely related *Dock1* and *Dock5* genes to study their function in mammals. We report that while *Dock5* is dispensable for normal mouse embryogenesis, *Dock1* has an essential role in embryonic development. A dramatic reduction of all skeletal muscle tissues is observed in *Dock1*-null embryos. Mechanistically, this embryonic defect is attributed to a strong deficiency in myoblast fusion, which is detectable both *in vitro* and *in vivo*. Furthermore, we have uncovered a contribution of *Dock5* toward myofiber development. These studies identify *Dock1* and *Dock5* as critical regulators of the fusion step during primary myogenesis in mammals and demonstrate that a specific component of the myoblast fusion machinery identified in *Drosophila* plays an evolutionarily conserved role in higher vertebrates.

Dock5 | mouse model | myogenesis | Myoblast City

The various developmental stages of mammalian myogenesis are characterized by mononucleated myoblasts fusing with each other and with existing myotubes to form syncytial muscle fibers (1). During embryogenesis, a first wave of myoblast fusion occurs to form primary fibers. At later fetal stages, myoblasts will undergo a second wave of fusion to form secondary fibers using the primary fibers as scaffolds (2). During adulthood, satellite cells, which are the muscle progenitor cells of the adult, fuse to existing myofibers to accomplish postnatal muscle growth and help regenerate injured tissues (3). Proper regulation of these various fusion events controls muscle fiber diameter and is central for appropriate contractile strength and muscle function (4). At the cellular level, myoblast fusion is divided into steps of cell–cell adhesion, alignment of cell membranes, and formation of membrane prefusion compartments, and the fusion is ultimately completed by the union of the two membranes (5). The molecular mechanisms regulating myoblast fusion in higher vertebrates remain poorly understood (6). The current understanding of myoblast fusion in mammals is largely derived from experiments with myoblast cell culture systems wherein the fusion step can be recapitulated *in vitro*. In contrast, myoblast fusion is a biological process prone to genetic analysis in *Drosophila*, and research over the last decade has suggested several candidate genes whose functions might be conserved in mammals (5). Surprisingly, none of these candidates have been confirmed thus far in mouse models.

The ease of visualization of muscle development in fly and zebrafish has greatly facilitated the identification and study of genes implicated in signaling cascades regulating myoblast fusion (5). Early in *Drosophila* development, myoblast precursors segregate into founder (fm) and fusion competent myoblasts (fcm) (5). Mechanistically, the fm attracts the fcm and thus serves as the nucleating entity during fiber formation. Receptors expressed in myoblasts known as Duf/Kirre, Rst, Sns, and Hbs connect the cell membranes of the interacting fm and fcm and activate pathways that ultimately converge on the modulation of the actin cytoskeleton (7–9).

In fm, Duf-Rst receptors recruit the intracellular Ants/Rols and Myoblast City (MBC) proteins to activate the Rac GTPase (10, 11). In parallel, Arf6 becomes activated by the guanine nucleotide exchange factor Loner, and this pathway is thought to spatially position the Rac GTPase for proper activation by MBC (12). In fcm, the Sns receptor couples to MBC to activate Rac, and in a parallel pathway Sns recruits the Solitary–Wasp complex to the cell membrane via the adapter protein Crk (13). The Solitary–Wasp pathway promotes the formation of actin patches involved in positioning Golgi-derived prefusion vesicles at the site of membrane fusion (13).

Zebrafish emerged as a potentially useful vertebrate genetic model for studying myoblast fusion *in vivo*. Zebrafish orthologue of the receptor Kirre, named Kirrel, was demonstrated to regulate myoblast fusion (14). Much like in *Drosophila*, Kirrel is thought to signal to the Rac GTPase to mediate fusion (14). However, in contrast to what was observed in flies, overexpression of Rac in myoblasts dramatically promoted myoblast fusion to form large syncytia, suggesting that Rac may regulate additional signaling cascades in fish myoblasts (14). Using morpholinos to interfere with gene function, Moore and colleagues (15) demonstrated an important role for *Dock1*, *Dock5*, *Crk*, and *Crkl* in myoblast fusion. Nonetheless, the relevance of these findings for mammalian myoblast fusion is not clear. For example, the closest related orthologue of Kirrel in mammals is a Nephrin-like protein (6). Nephrin has been studied in mammals and is implicated in kidney development (16). It thus remains to be demonstrated whether the machinery of myoblast fusion in fly and fish have a conserved role in mammals (6).

Genetic lesions in the *mbc* and *rac* genes lead to myoblast fusion defects in *Drosophila* (17, 18), and *mbc* acts upstream of the *rac* GTPase in this process (19). Mutation of *dElmo*, a MBC binding partner, was reported to disrupt myoblast fusion (20). In addition to its role in fusion, Rac activation by MBC/dELMO is critical in additional biological processes, including thorax closure, dorsal closure, phagocytosis, and border cell migration (21). MBC is the *Drosophila* orthologue of mammalian Dock1 (also known as Dock180) and *Caenorhabditis elegans* Ced-5, and these proteins are prototypical members of a novel superfamily of guanine nucleotide exchange factors for Rho GTPases

Author contributions: M.L., K.V., and J.-F.C. designed research; M.L., N.F., and J.-F.C. performed research; A.B. and A.H. contributed new reagents/analytic tools; M.L. and J.-F.C. analyzed data; and M.L., K.V., and J.-F.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

‖To whom correspondence may be addressed. E-mail: kvuori@burnham.org or jean-francois.cote@ircm.qc.ca.

This article contains supporting information online at www.pnas.org/cgi/content/full/0805546105/DCSupplemental.

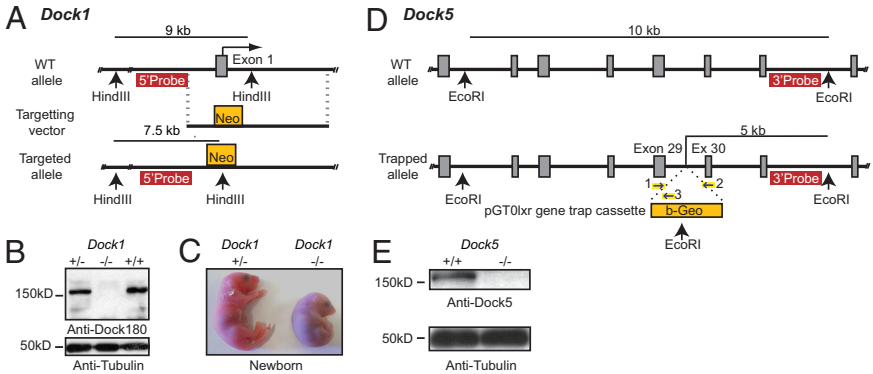

Fig. 1. Generation and analysis of *Dock1* and *Dock5* mutant mice. (*A*) Partial representation of the *Dock1* locus, the structure of the targeting vector, and the organization of the rearranged *Dock1* targeted allele. The probe used for Southern blot and the expected size of the HindIII fragments is indicated. (*B*) Western blot analysis showing the abrogation of protein expression in *Dock1*-null embryos. Antitubulin antibody was used to confirm equal loading of lysates. (*C*) Mice lacking *Dock1* expression die at birth with noninflated lungs (see also Fig. S1). (*D*) Partial representation of the *Dock5* locus surrounding exon 29 and the structure of the trapped locus. (*E*) Western blot analysis using anti-Dock5 antibody showing proper abrogation of the protein expression in the *Dock5* mutant. Antitubulin antibody was used to confirm equal loading of lysates.

(GEFs) (22–24). In mammals, Dock1, Dock2, and Dock5 proteins are members of the same subfamily of Dock1-related proteins. Dock1 is implicated in the control of Rac-mediated cell polarization, cell migration, phagocytosis of apoptotic cells, and fusion of myoblasts and macrophages *in vitro* (25–27). Additionally, interfering with *Dock1* and *Dock5* in zebrafish interfered with myoblast fusion (15).

We report that the formation of primary skeletal muscle fibers is severely impaired in *Dock1*-null animals. Our data demonstrate a central role for Dock1 in myoblast fusion *in vivo*. Genetic analyses further uncovered functional redundancy between *Dock1* and *Dock5* in myoblast fusion. We demonstrate a conserved function for a specific component of the *Drosophila* fusion-signaling pathway in mammalian myoblast fusion.

Results

Disruption of *Dock1* and *Dock5* Genes in Mice. Dock1 (also known as Dock180) and Dock5, two closely related Rac GEFs, are orthologues of *D. melanogaster* MBC and *C. elegans* Ced-5. To obtain insight into the *in vivo* roles of Dock1 and Dock5 in mammals, we generated mice with disrupted *Dock1* or *Dock5* loci by homologous recombination and gene trapping, respectively [Fig. 1 and detailed in supporting information (SI) *Text*]. Western blot analyses confirmed the proper elimination of the expression of the corresponding proteins in *Dock1*- and *Dock5*-mutant embryos (Fig. 1 *B* and *E*). Breeding of $Dock1^{+/-}$ mice demonstrated an essential role for *Dock1* during development as no viable $Dock1^{-/-}$ offspring was obtained (Table S1). Breeding of $Dock5^{+/-}$ mice established that homozygous mutant animals are viable (Table S1) and have no obvious morphological abnormality. *Dock1* mutant newborns were smaller, failed to straighten their body at birth, and became cyanotic within minutes after birth, possibly as a result of noninflated lungs (Fig. 1*C* and Fig. S1). These defects in breathing and posture are hallmarks of myogenesis defects and prompted us to perform characterization of the developing muscle in *Dock1* mutants.

Aberrant Muscle Development in the Absence of *Dock1*. We studied the embryonic expression profile of Dock1 and detected expression, both at the mRNA and protein levels, in the dermomyotome and myotome of developing embryonic day (E) 10.5 and E11.5 mice embryos, respectively (Fig. 2 *A* and *B* and Fig. S2). Although *Dock5* mRNA expression was not detectable at similar developmental stages by whole-mount *in situ* hybridization (data not shown), we detected the Dock5 protein in the myotome of E11.5 embryo by immunohistochemistry (Fig. 2*B*). Overall, these observations suggested that Dock1 and Dock5 could play an evolutionarily conserved role in mammalian myogenesis.

A systematic histological analysis of different muscle groups showed a dramatic and general reduction in muscle content in $Dock1^{-/-}$ embryos compared with $Dock1^{+/+}$ embryos at E16.5. We observed that the diaphragm was strikingly thinner and its attachment to the intercostal muscle was severely impaired in *Dock1* mutants (Fig. S3). Identical defects are already present and detectable at E14.5 (data not shown). Intercostal muscles

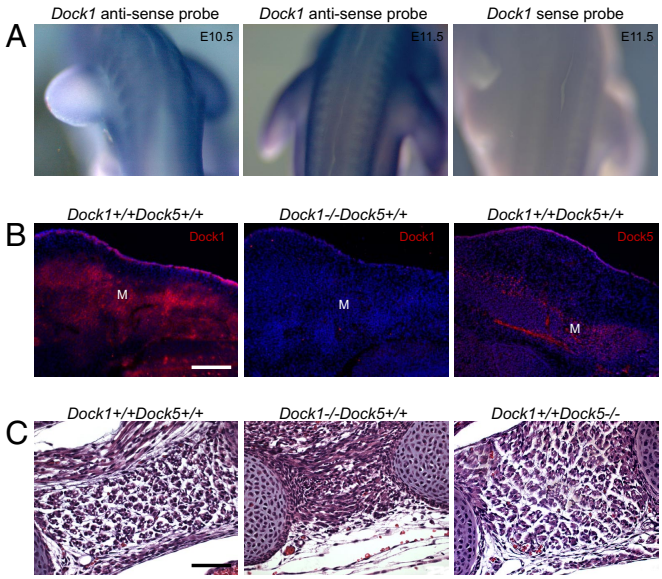

Fig. 2. Expression profile and characterization of the skeletal muscle in the absence of *Dock1* and *Dock5*. (*A*) Whole-mount RNA *in situ* hybridizations with *Dock1* antisense and sense probes demonstrate specific staining of *Dock1* in the dermomyotome of E10.5 and myotome of E11.5 embryos. (*B*) Immunohistochemistry with anti-Dock1 and anti-Dock5 antibodies demonstrated the presence of the proteins in the myotome and limb bud of E11.5 embryos. *Dock1*-null embryos were used to demonstrate the specificity of the primary antibody against Dock1. Nuclei are revealed by Hoechst staining (blue). M, myotome. (*C*) Sagittal sections through E16.5 WT and mutant *Dock1* and *Dock5* embryos stained with H&E. *Dock1*-null embryos display a severe reduction of their respiratory muscles. Mice lacking *Dock5* display normal muscle phenotype. (Magnifications: *A*, ×4; *B*, ×10; *C*, ×40.) (Scale bars: *B*, 250 μm; *C*, 75 μm.)

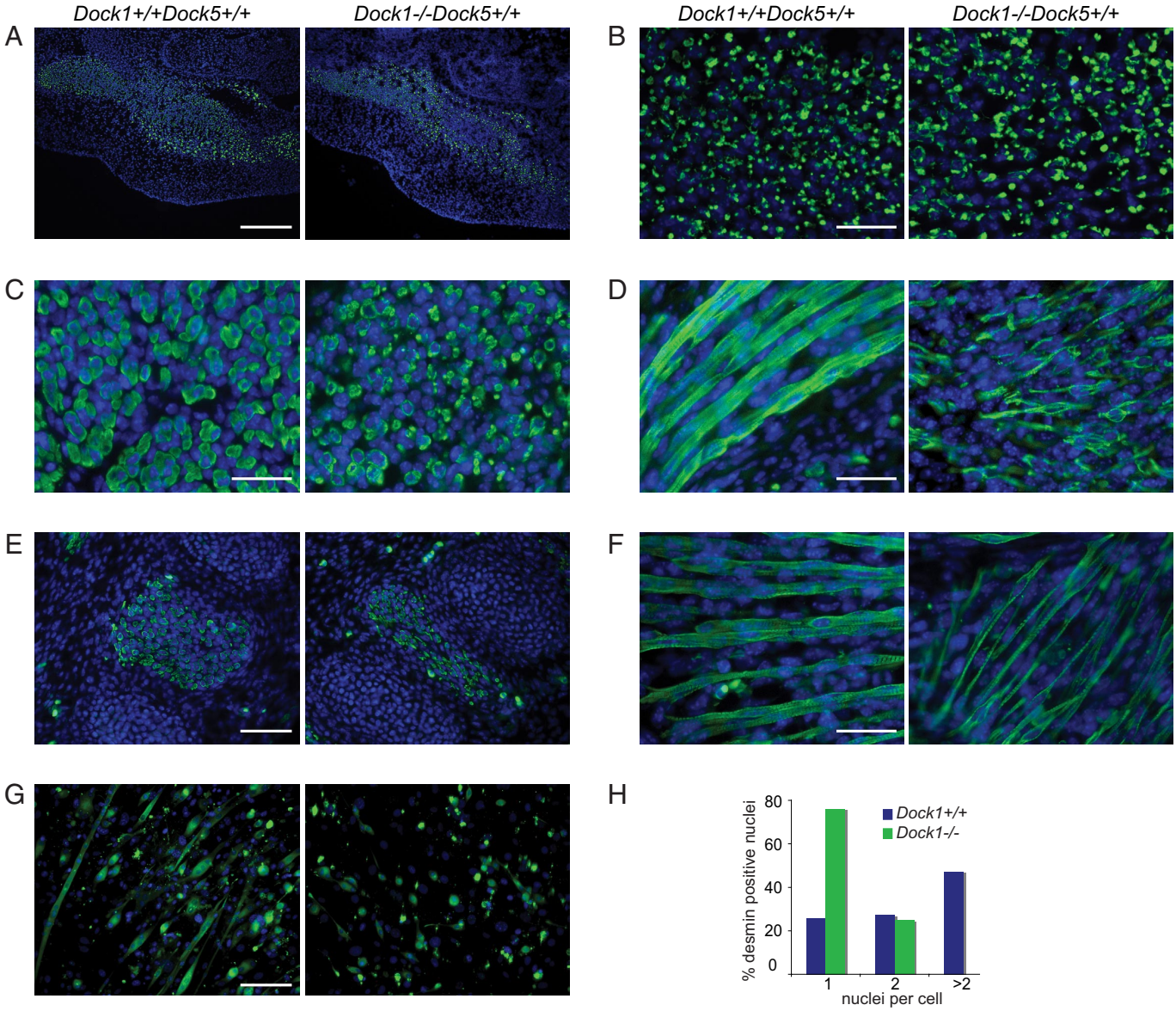

Fig. 3. The absence of *Dock1* affects myoblast fusion *in vivo*. (*A* and *B*) Cross section through E11.5 embryos stained with MF20 antibody (green) showing comparable myotomes (*A*) and fiber diameter (*B*) between WT and *Dock1*-null embryos. Nuclei are revealed by Hoechst staining (blue). (*C* and *D*) Cross-sections (*C*) and longitudinal sections (*D*) through E13.5 embryos stained with MF20 showing reduction in fibers diameter (*C*) in *Dock1*-null. Multinucleated fibers are present in *Dock1*$^{+/+}$ embryos but absent in *Dock1*-null animals (*D*). Nuclei are revealed by Hoechst staining (blue). (*E* and *F*) Sections through E14.5 WT and *Dock1* mutant embryos stained with MF20 showing reduction of the intercostal muscles. Longitudinal sections of intercostal muscles demonstrate the fusion defect (*F*). Nuclei are revealed by Hoechst staining (blue). (*G*) Primary myoblasts isolated from E18.5 WT and mutant embryos were used in an *in vitro* fusion assay. Cell fusion was evaluated by staining cells with an antidesmin antibody. Nuclei are revealed by DAPI staining. (*H*) Quantification of the experiment in *G*. (Magnifications: *A*, ×10; *B*, *E*, and *F*, ×40; *C*, *D*, and *G*, ×20.) (Scale bars: *A*, 250 μm; *B–D* and *F*, 30 μm; *E*, 60 μm; *G*, 130 μm.)

are also significantly reduced in size to the extent that ribs were observed to be stacked more closely together in *Dock1*$^{-/-}$ embryos (Fig. 2*C* and Fig. S3). As mentioned above, these malformations strongly suggest respiratory failure as a cause of death for *Dock1*-null animals. However, histological analyses of the lungs at E16.5 did not reveal any gross abnormalities in any of the genotypes studied (Fig. S3). Reduction in muscle mass was not only associated with respiratory muscles but was common in all skeletal muscle studied, including deep back muscles, tongue, and limb muscles (Fig. S3). Moreover, this reduction in muscle mass correlated with a striking reduction in fiber diameter at E18.5 (Fig. S3). In marked contrast, no obvious defects were noticeable in diaphragm and intercostal muscles of E16.5 *Dock5*$^{-/-}$ embryos (Fig. 2*C* and Fig. S3).

Impaired Myoblast Fusion *in Vivo* in *Dock1*-Null Embryos. We performed experiments to demonstrate that the establishment of myogenesis was normal in *Dock1*-null embryos (see *SI Text* and Figs. S4 and S5). Muscle development *in vivo* was further characterized at the molecular level by staining against the muscle-specific myosin heavy chain (MHC; MF20 antibody) to track the origin of the observed myogenesis defect. Although the diameter and number of myofibers were similar at E11.5 (Fig. 3 *A* and *B*), defects were found in *Dock1*-null muscles at E12.5 (data not shown) and E13.5 (Fig. 3 *C* and *D*). Reduction in muscle mass and in fusion became more prominent at E14.5 when compared with WT embryos (Fig. 3 *E* and *F*). Notably, we detected that most of the MF20-positive fibers aligned with one another but remained mononucleated in E13.5–E14.5 *Dock1*$^{-/-}$

embryos (Fig. 3 *D* and *F*). These results establish a role for Dock1 in primary myoblast fusion. To address specifically the requirement for Dock1 in the process of myoblast fusion, primary myoblasts were isolated from E18.5 $Dock1^{+/+}$ and $Dock1^{-/-}$ embryos. Upon selection and expansion of the primary cells in proliferation media, we tested the ability of desmin-positive myoblasts to undergo fusion *in vitro* and found that *Dock1*-null cells were unable to form long multinucleated fibers after 4 days in differentiating conditions in contrast to $Dock1^{+/+}$ myoblasts. (Fig. 3*G*). Quantification of the fusion index revealed that most of the mutant desmin-positive myoblasts remained mononucleated and a portion of the cells (≈20%) could form binucleated syncytia after differentiation (Fig. 3*H*). The presence of mononucleated fibers at these stages of development is highly reminiscent of the fusion defect observed in *mbc* mutants in *Drosophila*, suggesting an evolutionarily conserved role for *Dock1* in mammalian myoblast fusion.

Genetic Interaction Between *Dock1* and *Dock5* in Myogenesis. Because Dock5 expression pattern overlapped with Dock1 in the myotome, we tested the hypothesis that *Dock5* could also participate in myoblast fusion. To directly address this idea, we interbred $Dock1^{+/-}Dock5^{+/-}$ animals with the aim of studying muscle development in the progeny. No double-mutant embryos were recovered in this cross at E14.5 (Table S2). In parallel, we noted an increase of necrotic embryos, which likely represent double-mutant animals failing to undergo early embryogenesis. Embryos lacking one allele of *Dock1* in a $Dock5^{-/-}$ background, and conversely, embryos lacking one allele of *Dock5* in a $Dock1^{-/-}$ background were recovered and their muscle phenotype was analyzed.

Consistent with the histological data presented above (Fig. 2), MF20-positive fibers developed normally in intercostal muscles of $Dock5^{-/-}$ embryos (Fig. 4 *A* and *B*). Notably, double $Dock1^{+/-}Dock5^{+/-}$ heterozygous mutant animals also developed their muscles normally (Fig. 4*D*). Interestingly, $Dock1^{+/-}Dock5^{-/-}$ animals, which survived development, developed thinner myofibers (Fig. 4 *A*–*C*). This reduction in fiber thickness was, however, not completely penetrant because we observed a mixture of thin/thick fibers in some sections (data not shown). In $Dock1^{-/-}Dock5^{+/-}$ mutants, MF20-positive cells remained mononucleated as expected, but striking severe defects in MHC organization, cell elongation, and alignment were additionally uncovered (Fig. 4 *E* and *F*). Together, these data establish functional redundancy between *Dock1* and *Dock5* in the fine-tuning of myoblast fusion and fiber growth.

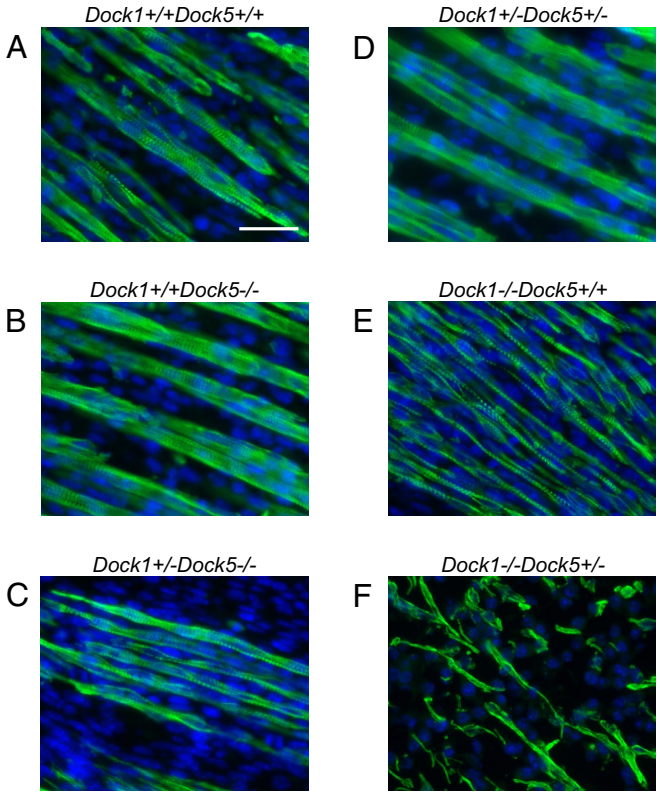

Fig. 4. Genetic interactions between *Dock1* and *Dock5* in muscle development. Sections of intercostal muscle of E14.5 embryos isolated from intercrosses of $Dock1^{+/-}Dock5^{+/-}$ animals were stained with MF20. Nuclei are revealed by Hoechst staining (blue). (*A*–*C*) While *Dock5* mutant animals have normal fibers (*A* and *B*), $Dock1^{+/-}$ $Dock5^{-/-}$ displayed thinner multinucleated fibers (*C*). (*D*) $Dock1^{+/-}Dock5^{+/-}$ mutants undergo normal muscle development. (*E* and *F*) $Dock1^{-/-}Dock5^{+/-}$ mutants display abnormal muscle development characterized by disorganized myosin heavy chain. (Magnification: ×40.) (Scale bar: 30 μm.)

Discussion

Genetic analyses in *Drosophila* have led to the understanding of some of the signaling cascades that regulate the fusion of myoblasts with each other and with developing myofibers, but it has remained unclear whether similar or different mechanisms operate *in vivo* in higher vertebrates (5, 6). One of the first genes identified to regulate myoblast fusion in *Drosophila* was *mbc* (17, 18). In this article, we investigate whether the mouse orthologues of *mbc*, namely *Dock1* and *Dock5*, are also involved in muscle development. *In vivo*, we demonstrate that while $Dock1^{-/-}$ myoblasts are specified properly, differentiated MF20-positive cells remain mononucleated at the time of primary myogenesis. In addition, we have uncovered a contribution of *Dock5* toward myofiber development. We conclude from these results that these GEFs play an evolutionarily conserved function at the step of myoblast fusion in the mouse.

A number of Rho-family GEFs have been analyzed by genetic means in mice. Surprisingly, only two Rho GEFs, *SOS1* and *TRIO*, are required for proper mammalian development (28). In the case of SOS1, mice die of placental defects likely attributed to impaired Ras activation (29). Whether the Rac GEF activity of SOS1 also contributes to embryogenesis remains to be investigated *in vivo*. *TRIO* mutants are also afflicted with myogenic development defects. However, these are phenotypically different from the ones reported here for *Dock1*. Briefly, myogenesis in *TRIO*-null animals appeared normal until E14.5, but significant defects in muscle fibers arise at E16.5. Interestingly, TRIO is a dual GEF for RhoA and Rac1; whether either one or both of these activities are important for muscle development is currently unknown. Recent data in C2C12 myoblasts *in vitro* suggest a major contribution of *TRIO* in the fusion process, raising the possibility that other GEFs can compensate for the loss of *TRIO in vivo* (30). More work will be required to fully appreciate the spatial and temporal activation of Rho GTPases in myoblast fusion. Together, these results suggest a role for *TRIO* in secondary myogenesis (28) while our work supports a key role for *Dock1* in primary myogenesis.

Recently, Pajcini *et al.* (27) reported that mammalian Dock1 is part of a common fusion machinery in myoblasts and in macrophages. In that study, siRNA-mediated knockdown of Dock1 interfered with, but did not block, fusion in C2C12 cells. It was suggested that incomplete knockdown of *Dock1* could explain why only a partial block in fusion was observed. Our data suggest the possibility that Dock5 is also expressed in C2C12 cells and could act redundantly with Dock1 in the fusion process. Pajcini *et al.* also proposed that silencing of Dock1 has no effect on cell proliferation but instead delayed cells from exiting the cell cycle after serum withdrawal. As a result, a delay in expression of myogenin and

MHC was observed in Dock1-silenced C2C12 cells in differentiation conditions. Likewise, we observed normal proliferation of *Dock1*-null desmin-positive myoblasts *in vivo* (Fig. S5). However, a delay in the apparition of MHC was not apparent *in vivo*. Remarkably, *ex vivo*, *Dock1*-null primary myoblasts also delayed their expression of MHC much like in C2C12 *in vitro* (data not shown). These observations highlight some of the limitations of *in vitro* analyses in myoblast fusion. While most *Dock1*-null cells remained mononucleated, we also observed binucleated cells in our *in vitro* fusion assay. This finding could represent cells undergoing cell division or, alternatively, it may suggest that *in vitro* myoblast–myoblast fusion is permissive but myoblast–fiber is not in *Dock1*-null cells.

Recent studies suggest a degree of similarity in the mechanisms of myoblast fusion between *Drosophila* and zebrafish (14, 15). In marked contrast to the results presented in this article, an equally important contribution for *Dock1* and *Dock5* in myoblast fusion was uncovered in zebrafish when morpholinos were used to knock down their expression. It is interesting to note that the splice morpholinos designed by Moore *et al.* (15) resulted in mRNA coding for the expression of truncated Dock1 and Dock5 proteins that contained the N-terminal ELMO-binding regions. Thus, it is possible that the experimentally generated mutant Dock1 and Dock5 proteins could act as dominant-negative proteins *in vivo* and therefore amplify the fusion phenotypes observed in Moore *et al.*'s study, thereby explaining the discrepancy with our results. However, the fact that we failed to observe the presence of $Dock1^{-/-}Dock5^{-/-}$ double mutants at E14.5 strongly suggests that these genes have redundant roles in fundamental biological processes. Here, we specifically investigated the contribution of these two genes in myogenesis and uncovered a functional redundancy in myoblast fusion. Most strikingly, in addition to remaining mononucleated, MF20-positive cells in $Dock1^{-/-}Dock5^{+/-}$ mutants displayed an abnormal morphology characterized by poorly organized MHC. Furthermore, they failed to elongate and align with each other. One possibility is that Dock5, redundant with Dock1, is playing a role in cytoskeletal organization. It will be important to clarify the exact contribution of *Dock5* and *Dock1* to muscle fiber formation.

It would not necessarily be surprising that *Dock1* and *Dock5* do not play entirely identical roles in mouse and fish. The muscles in fish and mouse are quite different. For example, myoblasts are specified in two populations in the zebrafish myotome (31). The "fast-twitch" are fusion competent cells that will generate classical syncytial myotubes in the myotome (14). The "slow-twitch" fibers are mononucleated and specified by *Sonic Hedgehog*. These mononucleated fibers are maintained fusion incompetent via the *u-boot* gene (31). While the myotomes of mouse and chick also develop initially from mononucleated fibers (32), to our knowledge, no equivalent of the zebrafish fusion incompetent slow-twitch have been identified in mammals. In *Drosophila*, there are two myoblast subpopulations known as the founder and fusion-competent cells (5). In fusion mutant flies, such as *mbc*-null, fusion-competent but not founder cells will undergo apoptosis and get cleared by macrophages (17). In mammals, such a concept of "leader" and "follower" myoblasts is not yet experimentally supported. Intriguingly, we observed at E13.5 that a subset of *Dock1*-null MF20-positive myoblasts undergoes apoptosis (Fig. S5). One hypothesis is that the apoptotic cells and the surviving cells could represent different populations of myoblasts, much like in *Drosophila*.

In conclusion, we observed a reduction of all skeletal muscles in *Dock1* mutants. We demonstrate that this embryonic defect is caused by a defect in myoblast fusion, which is detectable, both *in vitro* and *in vivo*. Genetic analyses further uncovered functional redundancy between *Dock1* and *Dock5* in myoblast fusion. These studies identify *Dock1* and *Dock5* as regulators of myoblast fusion during muscle development in mammals and demonstrate that a specific component of the machinery identified in *Drosophila* plays an evolutionarily conserved role in higher vertebrates.

Experimental Procedures

***Dock1* Mouse Knockout and *Dock5* Gene Trapping.** See *SI Text*.

Histology and Immunohistochemistry. For histology, embryos were fixed in 4% PFA, embedded in paraffin, sectioned, and stained with H&E. For immunohistochemistry analysis, embryos were embedded in OCT (Electron Corp.) and cryosectioned at 10 μm. For MF20 staining, an antigen retrieval technique was performed by using 10 mM citrate buffer, pH 6 according to standard procedures. Sections were blocked in PBS/0.2% Tween-20 (PBT) and 5% BSA for 1 h and incubated with primary antibody (MF20 1:20; Developmental Hybridoma) diluted in PBT and 5% BSA overnight. For detecting the Dock1 and Dock5 proteins, sections were blocked in PBS and 1% BSA and stained overnight in blocking buffer (anti-Dock180 1:100, C-19; Santa Cruz and rabbit polyclonal anti-Dock5 1:250). Sections were incubated with appropriate secondary antibodies for 1 h. Slides were mounted with Mowiol (VWR) reagent with Hoechst (Invitrogen).

Primary Myoblast Cell Cultures. Primary myoblasts were isolated from limbs of E18.5 mouse embryos from *Dock1* heterozygous crosses as described (33) and maintained in growth media [HAM's F-10 medium (Invitrogen) supplemented with 20% FBS and 2.5 ng/ml basic fibroblast growth factor (Invitrogen)]. For differentiation experiments, cells were plated and switched 4 days later to differentiation media [DMEM (Invitrogen) with 2% horse serum]. Differentiated myotubes were stained with anti-desmin antibody and DAPI.

See Table S3 for PCR primers used for different procedures.

ACKNOWLEDGMENTS. We thank Dr. M. Rudnicki (Ottawa Health Research Institute, Ottawa) for helpful discussions, reagents, and protocols and Drs. D. Hipfner and M. Kmita for critical reading of the manuscript. The MF20 antibody developed by Dr. D. Fischman was obtained from the Developmental Studies Hybridoma Bank (Iowa City, IA). This work was supported by a Canadian Institutes of Health Research operating grant (to J.-F.C.) and National Institutes of Health Grant CA102583 (to K.V.). M.L. was supported by a scholarship from the Canadian Institutes of Health Research. J.-F.C. holds a Canadian Institutes of Health Research New Investigator Award.

1. Horsley V, Pavlath GK (2004) Forming a multinucleated cell: Molecules that regulate myoblast fusion. *Cells Tissues Organs* 176:67–78.
2. Wigmore PM, Dunglison GF (1998) The generation of fiber diversity during myogenesis. *Int J* Dev Biol 42:117–125.
3. Sambasivan R, Tajbakhsh S (2007) Skeletal muscle stem cell birth and properties. *Semin Cell Dev Biol* 18:870–882.
4. Allen DL, Roy RR, Edgerton VR (1999) Myonuclear domains in muscle adaptation and disease. *Muscle Nerve* 22:1350–1360.
5. Chen EH, Grote E, Mohler W, Vignery A (2007) Cell-cell fusion. *FEBS Lett* 581:2181–2193.
6. Krauss RS (2007) Evolutionary conservation in myoblast fusion. *Nat Genet* 39:704–705.
7. Bour BA, Chakravarti M, West JM, Abmayr SM (2000) *Drosophila* SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. *Genes Dev* 14:1498–1511.
8. Ruiz-Gomez M, Coutts N, Price A, Taylor MV, Bate M (2000) *Drosophila* dumbfounded: A myoblast attractant essential for fusion. *Cell* 102:189–198.
9. Strunkelnberg M, *et al.* (2001) rst and its paralogue kirre act redundantly during embryonic muscle development in *Drosophila*. *Development* 128:4229–4239.
10. Chen EH, Olson EN (2001) Antisocial, an intracellular adaptor protein, is required for myoblast fusion in *Drosophila*. *Dev Cell* 1:705–715.
11. Rau A, *et al.* (2001) rolling pebbles (rols) is required in *Drosophila* muscle precursors for recruitment of myoblasts for fusion. *Development* 128:5061–5073.
12. Chen EH, Pryce BA, Tzeng JA, Gonzalez GA, Olson EN (2003) Control of myoblast fusion by a guanine nucleotide exchange factor, loner, and its effector ARF6. *Cell* 114:751–762.
13. Kim S, *et al.* (2007) A critical function for the actin cytoskeleton in targeted exocytosis of prefusion vesicles during myoblast fusion. *Dev Cell* 12:571–586.
14. Srinivas BP, Woo J, Leong WY, Roy S (2007) A conserved molecular pathway mediates myoblast fusion in insects and vertebrates. *Nat Genet* 39:781–786.
15. Moore CA, Parkin CA, Bidet Y, Ingham PW (2007) A role for the Myoblast city homologues Dock1 and Dock5 and the adaptor proteins Crk and Crk-like in zebrafish myoblast fusion. *Development* 134:3145–3153.
16. Patrakka J, Tryggvason K (2007) Nephrin: A unique structural and signaling protein of the kidney filter. *Trends Mol Med* 13:396–403.
17. Rushton E, Drysdale R, Abmayr SM, Michelson AM, Bate M (1995) Mutations in a novel gene, myoblast city, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development* 121:1979–1988.

18. Erickson MR, Galletta BJ, Abmayr SM (1997) *Drosophila* myoblast city encodes a conserved protein that is essential for myoblast fusion, dorsal closure, and cytoskeletal organization. *J Cell Biol* 138:589–603.
19. Nolan KM, *et al.* (1998) Myoblast city, the *Drosophila* homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. *Genes Dev* 12:3337–3342.
20. Geisbrecht ER, *et al.* (2008) *Drosophila* ELMO/CED-12 interacts with Myoblast city to direct myoblast fusion and ommatidial organization. *Dev Biol* 314:137–149.
21. Cote JF, Vuori K (2007) GEF what? Dock180 and related proteins help Rac to polarize cells in new ways. *Trends Cell Biol* 17:383–393.
22. Cote JF, Vuori K (2002) Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. *J Cell Sci* 115:4901–4913.
23. Brugnera E, *et al.* (2002) Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. *Nat Cell Biol* 4:574–582.
24. Meller N, Irani-Tehrani M, Kiosses WB, Del Pozo MA, Schwartz MA (2002) Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. *Nat Cell Biol* 4:639–647.
25. Cote JF, Motoyama AB, Bush JA, Vuori K (2005) A novel and evolutionarily conserved PtdIns(3,4,5)P3-binding domain is necessary for DOCK180 signaling. *Nat Cell Biol* 7:797–807.
26. Grimsley CM, *et al.* (2004) Dock180 and ELMO1 proteins cooperate to promote evolutionarily conserved Rac-dependent cell migration. *J Biol Chem* 279:6087–6097.
27. Pajcini KV, Pomerantz JH, Alkan O, Doyonnas R, Blau HM (2008) Myoblasts and macrophages share molecular components that contribute to cell–cell fusion. *J Cell Biol* 180:1005–1019.
28. O'Brien SP, *et al.* (2000) Skeletal muscle deformity and neuronal disorder in Trio exchange factor-deficient mouse embryos. *Proc Natl Acad Sci USA* 97:12074–12078.
29. Wang DZ, *et al.* (1997) Mutation in Sos1 dominantly enhances a weak allele of the EGFR, demonstrating a requirement for Sos1 in EGFR signaling and development. *Genes Dev* 11:309–320.
30. Charrasse S, *et al.* (2007) M-cadherin activates Rac1 GTPase through the Rho-GEF trio during myoblast fusion. *Mol Biol Cell* 18:1734–1743.
31. Roy S, Wolff C, Ingham PW (2001) The u-boot mutation identifies a Hedgehog-regulated myogenic switch for fiber-type diversification in the zebrafish embryo. *Genes Dev* 15:1563–1576.
32. Hollway GE, Currie PD (2003) Myotome meanderings. Cellular morphogenesis and the making of muscle. *EMBO Rep* 4:855–860.
33. Sabourin LA, Girgis-Gabardo A, Seale P, Asakura A, Rudnicki MA (1999) Reduced differentiation potential of primary MyoD−/− myogenic cells derived from adult skeletal muscle. *J Cell Biol* 144:631–643.