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Activation of the myogenin promoter during mouse embryogenesis in the absence of positive autoregulation

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ABSTRACT **Myogenin, a member of the MyoD family of helix–loop–helix proteins, can induce myogenesis in a wide range of cell types. In addition to activating muscle structural genes, members of the MyoD family can autoactivate their own and cross-activate one another's expression in transfected cells. This has led to the hypothesis that autoregulatory loops among these factors provide a mechanism for amplifying and maintaining the muscle-specific gene expression program *in vivo*. Here, we make use of myogenin-null mice to directly test this hypothesis. To investigate whether the myogenin protein autoregulates the myogenin gene during embryogenesis, we introduced a myogenin–*lacZ* transgene into mice harboring a null mutation at the myogenin locus. Despite a severe deficiency of skeletal muscle in myogenin-null neonates, the myogenin–*lacZ* transgene was expressed normally in myogenic cells throughout embryogenesis. These results show that myogenin is not required for regulation of the myogenin gene and argue against the existence of a myogenin autoregulatory loop in the embryo.**

Positive autoregulation has been invoked as a mechanism through which several genes that regulate cell fate induce and maintain their own expression (1–4). Thus, the discovery that members of the MyoD family (MyoD, myogenin, Myf5, and MRF4) can autoactivate their own and cross-activate one another's expression in transfected cells led to the notion that such autoregulatory loops might provide a mechanism for amplifying the expression of these genes above the threshold required to initiate myogenesis and for stabilizing the muscle-specific gene expression program (5–8). Whether these autoregulatory interactions occur during embryogenesis or are simply a tissue culture phenomenon remains to be determined.

Myogenin is the only member of the MyoD family that is expressed in all skeletal muscle cells (9, 10). During embryogenesis, myogenin gene expression is detected within myogenic cells in the somitic myotomes and the limb buds and subsequently within differentiated skeletal muscle fibers throughout the body (11–15). In tissue culture cells, myogenin expression is rapidly upregulated when myoblasts enter into the differentiation pathway in response to withdrawal of growth factors. In contrast, MyoD and Myf5 are expressed in proliferating myoblasts prior to differentiation (16, 17) and MRF4 is expressed during myofiber maturation (8, 18, 19).

Gene targeting in transgenic mice has shown that myogenin is essential for muscle development (20, 21) and that its functions are distinct from those of MyoD and Myf5 (refs. 22 and 23; reviewed in ref. 24). In the absence of myogenin, muscle-forming regions of neonatal mice are populated by cells that express MyoD but not most muscle structural genes. The expression of MyoD indicates that these cells are committed to the myogenic lineage, but the precise point in the myogenic pathway at which they are arrested is unclear.

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To investigate whether the myogenin protein positively autoregulates the myogenin gene during embryogenesis and to further define the properties of myogenin-null myoblasts, we examined the expression of a myogenin–*lacZ* transgene (13) in mice homozygous for a null mutation of the myogenin gene. Here we report that the myogenin gene promoter is expressed in the correct temporospatial pattern throughout embryogenesis of myogenin-null mice. These results demonstrate that the myogenin protein is not required to initiate or to maintain expression of the myogenin gene and argue against the existence of a myogenin positive autoregulatory loop in the embryo.

MATERIALS AND METHODS

Breeding Transgenic Mice. The myogenin–*lacZ* transgene, *Myo1565lacZ*, has been described previously (12, 13). Homozygosity of the transgene was confirmed by breeding transgenic mice to nontransgenic mice and finding that all offspring express LacZ (β-galactosidase). The transgene showed an identical expression pattern in the homozygous and heterozygous states. (C57BL/6 × CBA)F_1 mice were used for all crosses. The myogenin-null mutation contains a neomycin-resistance gene inserted into the first exon (20). The null allele was detected by Southern analysis of genomic DNA digested with *Bam*HI and hybridized to a *Sac* I–*Pst* I fragment located at the 5′ end of the *neo* insertion. The targeted and wild-type myogenin alleles give rise to 8.0- and 8.5-kb bands, respectively.

Histology. Embryos were isolated, fixed and stained for LacZ activity as described (12, 13). Briefly, embryos were fixed in 2% paraformaldehyde/0.2% glutaraldehyde in phosphate-buffered saline (PBS; 140 mM NaCl/10 mM sodium phosphate, pH 7.2) at 4°C, rinsed, and stained with X-Gal staining solution (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside at 1 mg/ml/2 mM $MgCl_2$/5 mM $K_3Fe(CN)_6$/5 mM $K_4Fe(CN)_6$ in PBS) at room temperature overnight. They were then cleared in PBS for 10 h and stored in 4% formaldehyde.

Whole-mounted embryos were dehydrated with ethanol, cleared in xylene, and embedded in paraffin. Embryos were sectioned transversely on a microtome. Serial sections were cut to a thickness of 5 μm and counterstained with hematoxylin and eosin.

Tongues from newborn mice were removed and immersed in 0.2 M sucrose at 4°C overnight. The tongues were then quickly frozen in optimal cutting temperature compound (OCT; Miles) and sectioned to a thickness of 5 μm in a cryostat. Sections were maintained frozen before fixation and staining. Frozen sections were fixed with 2% paraformaldehyde/0.2% glutaraldehyde in PBS for 30 sec at room temperature, rinsed twice with PBS and stained for LacZ activity with X-Gal staining solution as described above. Sections were then counterstained with hematoxylin and eosin.

Abbreviations: p.c., postcoitum; bHLH, basic helix–loop–helix.
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RESULTS

Introduction of the Myogenin–*lacZ* Transgene into Myogenin-Null Mice. To determine whether transcriptional activity of the myogenin promoter requires myogenin protein, we introduced a myogenin–*lacZ* transgene into mice carrying a null mutation in the myogenin gene. The mutation within the myogenin locus results in the absence of myogenin protein in the homozygous state, causing a severe deficiency of skeletal muscle at birth (20). The heterozygous myogenin-null mutation results in half the normal amount of myogenin protein but has no obvious effect on muscle development or viability. As a marker for myogenin transcription, we used the myogenin–*lacZ* transgene, *Myo1565lacZ*, which contains the DNA sequence from nucleotides −1565 to +18 relative to the myogenin transcription initiation site linked to *lacZ*. This reporter gene is expressed in the same spatial and temporal pattern as the endogenous myogenin gene and serves as a faithful marker of myogenin transcription throughout embryogenesis (12, 13).

The mating strategy used to obtain myogenin-null embryos harboring *Myo1565lacZ* is shown in Fig. 1. First, mice homozygous for *Myo1565lacZ* were crossed with mice heterozygous for the myogenin-null mutation, and female offspring that were hemizygous for the *Myo1565lacZ* transgene and heterozygous for myogenin [Z^+/Z^-, *myo*(+/−)] were obtained. These mice were then crossed with mice homozygous for *Myo1565lacZ* (Z^+/Z^+) to obtain male offspring homozygous for the transgene and heterozygous for myogenin [Z^+/Z^+, *myo*(+/−)]. Finally, these males were crossed with females that were heterozygous for myogenin and did not carry the transgene. Since the male mice used in the final cross were homozygous for *Myo1565lacZ*, all offspring of this cross carried the transgene, thereby allowing transgene expression to be compared among the three genetic backgrounds, *myo*(+/+), *myo*(+/−), and *myo*(−/−), in the same litters. Before beginning the crosses, we carefully examined the expression pattern of *Myo1565lacZ* in wild-type mice homozygous for the transgene to ensure that its homozygosity did not result in a phenotype as a consequence of a random insertion of the transgene into an essential gene. Mice carrying the homozygous transgene were indistinguishable from wild-type mice.

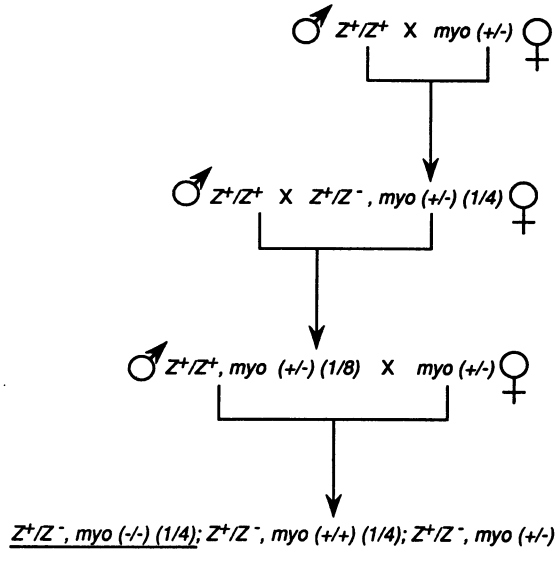

FIG. 1. Strategy to obtain myogenin-null mice carrying *Myo1565lacZ*. Male mice homozygous for the transgene *Myo1565lacZ* (Z^+/Z^+) were crossed with female mice heterozygous for the null mutation at the myogenin locus *myo*(+/−). One-fourth of the offspring were hemizygous for the transgene and heterozygous for the myogenin null allele [Z^+/Z^-, *myo*(+/−)]. Females of this genotype were crossed with Z^+/Z^+ males. One-eighth of the offspring were homozygous for the transgene and heterozygous for the myogenin-null mutation [Z^+/Z^+, *myo*(+/−)]. Males of this genotype were crossed to *myo*(+/−) females. All offspring from this final cross carried the *Myo1565lacZ* transgene. The ratios of the three genotypes in the final litters are indicated in parentheses. The genotype of the myogenin-null embryos harboring *Myo1565lacZ* is underlined.

Normal Expression of Myogenin–*lacZ* in Myogenin-Null Embryos. The expression pattern of *Myo1565lacZ* in wild-type and myogenin (−/−) embryos was compared beginning at day

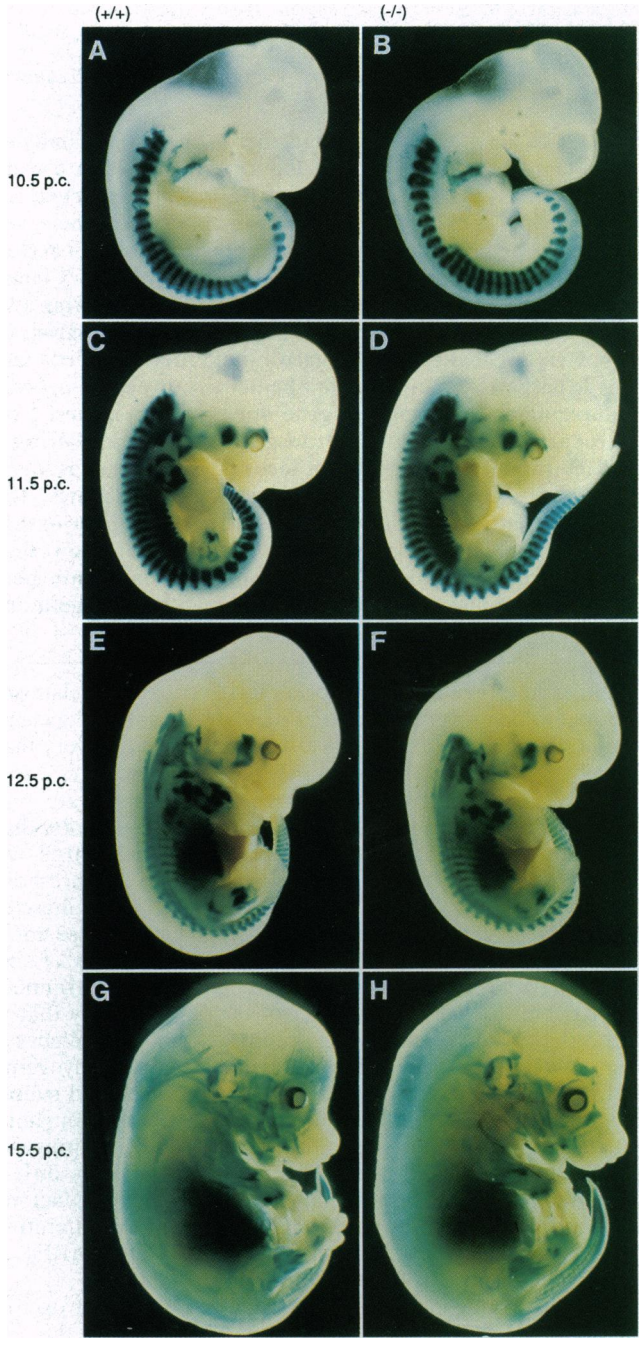

FIG. 2. Expression of the myogenin–*lacZ* transgene in wild-type and myogenin-null mouse embryos. Wild-type and myogenin-null embryos harboring the myogenin–*lacZ* transgene were stained for LacZ activity. (*A*, *C*, *E*, and *G*) Wild-type embryos. (*B*, *D*, *F*, and *H*) Myogenin-null embryos. (*A* and *B*) Day 10.5 p.c.; (*C* and *D*) day 11.5 p.c.; (*E* and *F*) day 12.5 p.c.; (*G* and *H*) day 15.5 p.c. Myogenin-mutant embryos were distinguishable from wild-type embryos at day 15.5 p.c. because of fluid accumulation at the base of the skull. The skin of the day 15.5 p.c. embryos was removed before the embryos were photographed. Genotypes of all embryos were determined by Southern analysis of yolk sac DNA.

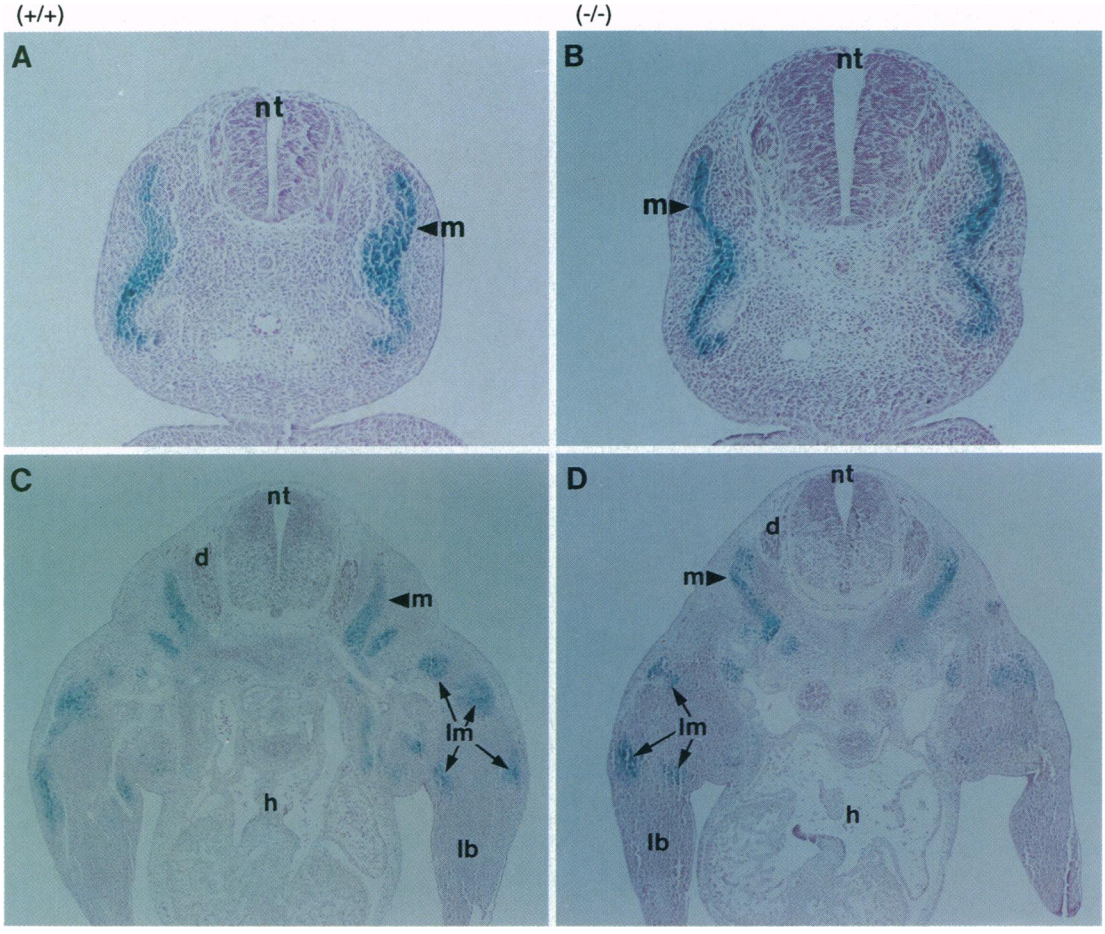

FIG. 3. Transverse sections of day 11.5 p.c. mouse embryos harboring the myogenin–*lacZ* transgene. Transgenic embryos were stained for LacZ expression and transverse sectioned through the caudal somites (approximately somite 30) (*A* and *B*) or through the forelimb bud and thoracic somites (approximately somite 13) (*C* and *D*). Sections were stained with hematoxylin and eosin. The myogenin–*lacZ* transgene was expressed specifically in the myotome and in muscle-forming regions of the limb buds and trunk. There was no detectable difference in the spatial pattern of expression of the transgene between wild-type and myogenin-mutant embryos. (*A* and *C*) Wild-type. (*B* and *D*) Myogenin mutant. Genotypes of embryos were determined by Southern analysis of yolk sac DNA. d, dorsal root ganglion; h, heart; lb, forelimb bud; lm, forming limb musculature; m, myotome; nt, neural tube.

10.5 postcoitum (p.c.). As reported previously (20, 21), the myogenin–*lacZ* transgene was expressed in the first 30 somites by day 10.5 p.c., but it was not expressed in the limb buds at this stage (Fig. 2*A*). By day 11.5 p.c, LacZ expression extended to the last somite and was also observed in myogenic cells within the limb buds and branchial arches (Fig. 2*C*). By day 12.5 p.c, expression of the transgene intensified in the forming skeletal muscle throughout the trunk, limbs, and face (Fig. 2*E*). At all of these stages, the expression pattern of the myogenin–*lacZ* transgene in myogenin-null embryos (Fig. 2 *B*, *D*, and *F*) was indistinguishable from that in wild-type embryos (Fig. 2 *A*, *C*, and *E*).

To examine the pattern of myogenin gene transcription in more detail, transgene expression was visualized at the single-cell level in transverse sections through the somites and limb buds of day 11.5 p.c. embryos. In both the wild-type and the myogenin-mutant embryos, LacZ expression was observed in the somitic myotomes and in muscle-forming regions of the limbs (Fig. 3). Remarkably, there was no significant difference in the spatial pattern or intensity of myogenin–*lacZ* expression in the wild-type and mutant embryos at this or earlier stages (Fig. 3 and data not shown). Thus, despite the near absence of skeletal muscle in myogenin-null mice at birth (20, 21), the myotome appeared to develop correctly in myogenin-null embryos. The migration of myogenic precursors to the limb also appeared to proceed normally in the absence of myogenin.

By day 15.5 p.c., wild-type and mutant embryos showed LacZ staining in developing muscles throughout the body (Fig. 2 *G* and *H*). The LacZ staining pattern of embryos at day 15.5 p.c. demonstrated that individual muscles were correctly patterned in the absence of myogenin, suggesting that muscle attachment sites and formation of muscle fascia do not depend on muscle fiber maturation, which is clearly aberrant in the mutant mice (20, 21).

Expression of the Myogenin–*lacZ* Transgene in Differentiation-Defective Myoblasts. The muscle of myogenin-null neonates predominantly contains disorganized, mononucleated cells and only a small number of differentiated myotubes (20, 21). Because the muscles of myogenin-null mice appeared to be correctly patterned, judging from LacZ expression, it was of interest to determine whether the myogenin–*lacZ* transgene was expressed in the subpopulation of differentiated cells or in mononucleated cells arrested along the differentiation pathway. We therefore examined LacZ expression in thin sections of the tongues of mutant neonates. Whereas LacZ staining in the tongues of wild-type neonates was confined to multinucleated muscle fibers, LacZ staining was observed in the differentiation-defective, mononucleated cells from myogenin-null neonates at levels comparable to that of differentiated muscle fibers from wild-type littermates (Fig. 4). Similar staining was observed in mononucleated cells within the limbs and diaphragm of myogenin-null mice (data not shown).

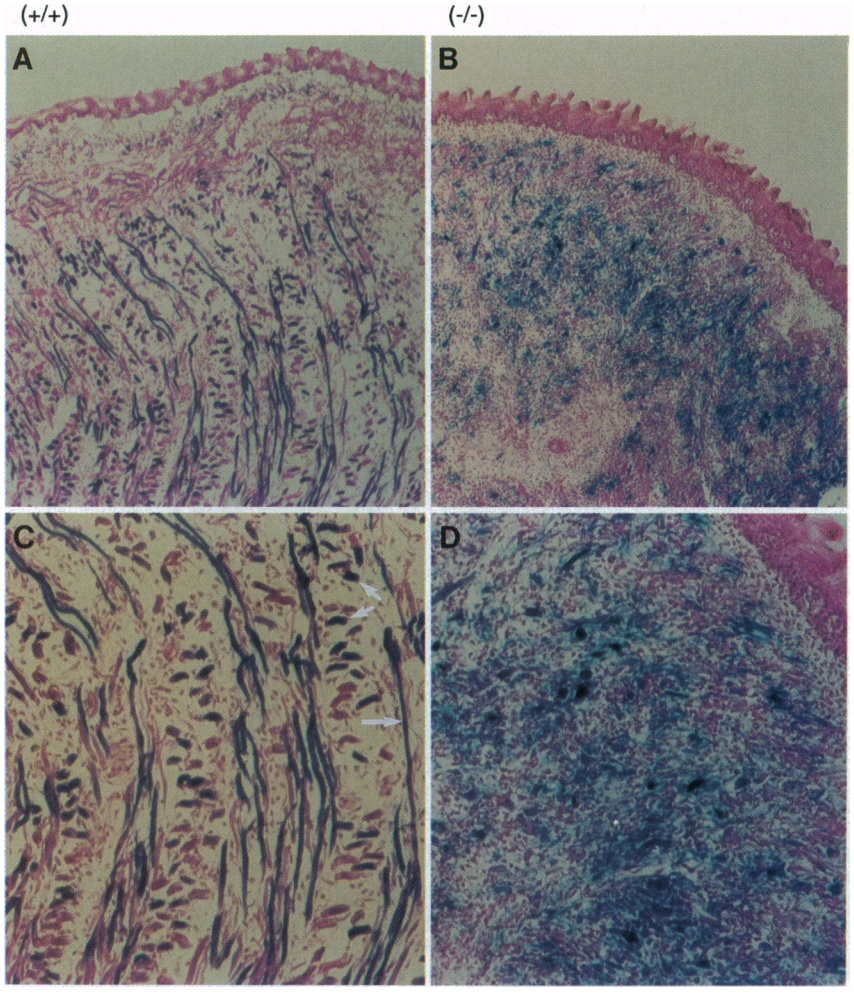

FIG. 4. Sections of tongue of neonatal mice harboring the myogenin–*lacZ* transgene. Sections were cut through the tongues of wild-type and myogenin-null neonates and were stained for LacZ expression. (*A* and *C*) Wild-type. (*B* and *D*) Myogenin mutant. (*A* and *B*, ×62; *C* and *D*, ×124.) LacZ expression in muscle fibers is observed in wild-type neonates. In myogenin-mutant neonates, comparable levels of staining can be seen in mononucleated cells, but few muscle fibers are detectable. Muscle fibers in sections from wild-type neonates are oriented perpendicularly, with some being longitudinal (long arrow in *C*) and others being transverse (short arrows in *C*). Genotypes of neonates were determined by Southern analysis of yolk sac DNA.

DISCUSSION

In light of the autoregulatory activity of myogenic basic helix–loop–helix (bHLH) proteins in transfected cells (5–8), it was reasonable to anticipate that myogenin might amplify or maintain its own expression during embryogenesis. Consistent with this notion was the finding that either of two E-boxes in the myogenin gene promoter is essential for expression of a myogenin–*lacZ* transgene in somites and limb buds of transgenic mice (13, 14). Nevertheless, our results demonstrate that activation of myogenin gene expression during embryogenesis occurs normally in the absence of the myogenin protein. Thus, although myogenin can induce expression of its own gene in transfected cells (5–7), it does not appear that this type of autoregulatory loop is essential in the regulation of myogenin gene expression in the embryo. The normal pattern of activation of the myogenin promoter in the somites, limb buds, and other muscle-forming regions of myogenin-null embryos also indicates that the regulatory cues leading to myogenin gene expression are operative in myogenic cells that are blocked in the differentiation pathway.

Considering the near absence of differentiated skeletal muscle in myogenin-null neonates, it is striking that the myogenin–*lacZ* transgene was expressed normally in the somitic myotomes and limb buds of myogenin-null embryos. These results are consistent with recent findings that certain muscle structural genes are expressed in the myotomes of myogenin-null mice up to day 12.5 p.c. but subsequent muscle development is arrested (38).

Activation of the myogenin gene promoter in the somites and limb buds was previously shown to require an E-box, to which members of the MyoD family bind, and a binding site for members of the myocyte enhancer factor-2 (MEF2) family of the MCM1, Agamous, Deficiens, Serum response factor (MADS) proteins (13, 14). MEF2 expression can be induced by myogenin and other myogenic bHLH proteins in transfected cells (25, 26). During embryogenesis, MEF2 gene expression is initiated in the somites and limb buds after the expression of myogenin (27), which suggests that MEF2 may function in a positive-feedback loop to amplify and maintain myogenin expression. The normal expression of the myogenin–*lacZ* transgene in somites and limb buds of myogenin-null embryos suggests that activation of MEF2 expression in these myogenic cells is not dependent on myogenin. Indeed, MEF2 transcripts show normal temporal and spatial regulation in myogenin-null embryos (A. Rawls and E.N.O., unpublished results).

Since the myogenin gene does not appear to be a target for regulation by the myogenin protein in the embryo, the bHLH factor most likely to activate transcription through the E-boxes in the myogenin promoter is Myf5, which is expressed immediately prior to myogenin during embryogenesis (28, 29). Myf5

is first expressed in the dermamyotome on day 8 p.c. (28), and is followed by myogenin a half day later. MRF4 is expressed transiently on day 9.0 p.c., and MyoD expression begins on day 10.5 p.c. (30, 31). It should be pointed out that there has been disagreement concerning the expression pattern of myogenin protein in the embryo. Cusella-DeAngelis *et al.* (32) reported that myogenin protein does not appear in the somites until day 10.5 p.c., which is two days later than the initial expression of myogenin mRNA. In contrast, Smith *et al.* (33) found a temporal concordance between myogenin mRNA and protein expression.

There appears to be an intimate homeostatic relationship among the myogenic bHLH genes, such that alteration in the expression of one can change the relative level of expression of one or more of the others. For example, *MyoD* and *Myf5* appear to be reciprocally regulated; in the absence of MyoD, Myf5 expression is upregulated and vice versa (22, 23, 34). The reduction in MRF4 expression in myogenin-null neonates also suggests that the late phase of MRF4 expression is dependent directly or indirectly on myogenin (20).

Given the changes in expression of the myogenic bHLH genes when one member of the family is removed from the network, one might have anticipated that removing myogenin would alter the activity of the myogenin gene by altering the expression of other members of the family at some stage of development or by eliminating a myogenin autoregulatory loop. In fact, our results suggest that the myogenin gene is insulated from the myogenin protein and from potential fluctuations its absence might induce in the levels of expression of other members of the family. Now that the control regions of the other myogenic bHLH genes are being identified (35–37), it should be possible to determine whether they are targets for autoregulatory loops during muscle development and to further define the potential hierarchical relationships among these genes.

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1. Bienz, M. & Tremml, G. (1988) *Nature (London)* **333,** 576–578.
2. Hiromi, Y. & Gehring, W. J. (1987) *Cell* **50,** 963–974.
3. Regulski, M., Dessain, N., McGinnis, N. & McGinnis, W. (1991) *Genes Dev.* **5,** 278–286.
4. Rhodes, S. J., Chen, R., DiMattia, G. E., Scully, K. M., Kalla, K. A., Lin, S.-C., Yu, V. C. & Rosenfeld, M. G. (1993) *Genes Dev.* **7,** 913–932.
5. Thayer, M. J., Tapscott, S. J., Davis, R. L., Wright, W. E., Lassar, A. B. & Weintraub, H. (1989) *Cell* **58,** 241–248.
6. Braun, T., Bober, E., Buschhausen-Denker, G., Kotz, S., Grzeschik, K. & Arnold, H. H. (1989) *EMBO J.* **8,** 3617–3625.
7. Edmondson, D. G., Cheng, T.-C., Cserjesi, P., Chakraborty, T. & Olson, E. N. (1992) *Mol. Cell. Biol.* **12,** 3665–3677.
8. Miner, J. H. & Wold, B. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1089–1093.
9. Wright, W. E., Sassoon, D. A. & Lin, V. K. (1989) *Cell* **56,** 607–617.
10. Edmondson, D. G. & Olson, E. N. (1989) *Genes Dev.* **3,** 628–640.
11. Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lassar, A. B., Weintraub, H. & Buckingham, M. (1989) *Nature (London)* **341,** 303–307.
12. Cheng, T.-C., Hanley, T. A., Mudd, J., Merlie, J. P. & Olson, E. N. (1992) *J. Cell Biol.* **119,** 1649–1656.
13. Cheng, T.-C., Wallace, M., Merlie, J. P. & Olson, E. N. (1993) *Science* **261,** 215–218.
14. Yee, Y.-T. & Rigby, P. W. J. (1993) *Genes Dev.* **7,** 1277–1289.
15. Fujisawa-Sehara, A., Hanaoka, K., Hayasaka, M., Hiromasa-Yagami, T. & Nabeshima, Y. (1993) *Biochem. Biophys. Res. Commun.* **191,** 351–356.
16. Davis, R. L., Weintraub, H. & Lassar, A. B. (1987) *Cell* **51,** 987–1000.
17. Braun, T., Buschhausen-Denker, G., Bober E., Tannich, E. & Arnold, H. H. (1989) *EMBO J.* **8,** 701–709.
18. Rhodes, S. J. & Konieczny, S. F. (1989) *Genes Dev.* **3,** 2050–2061.
19. Braun, T., Bober, E., Winter, B., Rosenthal, N. & Arnold, H. H. (1990) *EMBO J.* **9,** 821–831.
20. Hasty, P., Bradley, A., Morris, J. H., Edmondson, D. G., Venuti, J. M., Olson, E. N. & Klein, W. H. (1993) *Nature (London)* **364,** 501–506.
21. Nabeshima, Y., Hanaoka, K., Hayasaka, M., Esumi, E., Li, S., Nanoka, I. & Nabeshima, Y. (1993) *Nature (London)* **364,** 532–535.
22. Rudnicki, M. A., Braun, T., Hinuma, S. & Jaenisch, R. (1992) *Cell* **71,** 383–390.
23. Braun, T., Rudnicki, M. A., Arnold, H. H. & Jaenisch, R. (1992) *Cell* **71,** 369–382.
24. Olson, E. N. & Klein, W. H. (1994) *Genes Dev.* **8,** 1–8.
25. Cserjesi, P. & Olson, E. N. (1991) *Mol. Cell. Biol.* **11,** 4854–4862.
26. Lassar, A. B., Davis, R. L., Wright, W. E., Kadesch, T., Murre, C., Voronova, A., Baltimore, D. & Weintraub, H. (1991) *Cell* **66,** 305–315.
27. Edmondson, D. G., Lyons, G. E., Martin, J. F. & Olson, E. N. (1994) *Development (Cambridge, U.K.)* **120,** 1251–1263.
28. Ott, M.-O., Bober, E., Lyons, G., Arnold, H. H. & Buckingham, M. (1991) *Development (Cambridge, U.K.)* **111,** 1097–1111.
29. Buckingham, M. (1992) *Trends Genet.* **8,** 144–148.
30. Hinterberger, T. J., Sassoon, D. A., Rhodes, S. J. & Konieczny, S. F. (1991) *Dev. Biol.* **147,** 144–156.
31. Bober, E., Lyons, G. E., Braun, T., Cossu, G., Buckingham, M. & Arnold, H. H. (1991) *J. Cell Biol.* **113,** 1225–1265.
32. Cusella-DeAngelis, M., Lyons, G., Sonnin, C., DeAngelis, L., Vivarelli, E., Farmer, K., Wright, W., Molinaro, M., Bouche, M., Buckingham, M. & Cossu, G. (1992) *J. Cell Biol.* **116,** 1243–1255.
33. Smith, T. H., Kachinsky, A. M. & Miller, J. B. (1994) *J. Cell Biol.* **127,** 95–105.
34. Peterson, C. A., Gordon, H., Hall, Z. W., Paterson, B. M. & Blau, H. M. (1990) *Cell* **62,** 493–502.
35. Tapscott, S. J., Lassar, A. B. & Weintraub, H. (1992) *Mol. Cell. Biol.* **12,** 4994–5003.
36. Goldhamer, D. J., Faerman, A., Shani, M. & Emerson, C. P., Jr. (1992) *Science* **256,** 538–542.
37. Patapoutian, A., Miner, J. H., Lyons, G. E. & Wold, B. (1993) *Development (Cambridge, U.K.)* **118,** 61–69.
38. Venuti, J. M., Morris, J. H., Vivian, J. L., Olson, E. N. & Klein, W. H. (1995) *J. Cell Biol.*, in press.