Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 10632–10636, September 1998
Developmental Biology

MLL, a mammalian *trithorax*-group gene, functions as a transcriptional maintenance factor in morphogenesis

BENJAMIN D. YU, ROBIN D. HANSON, JAY L. HESS, SUSAN E. HORNING, AND STANLEY J. KORSMEYER*

Howard Hughes Medical Institute, Division of Molecular Oncology, Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, MO 63110

Contributed by Stanley J. Korsmeyer, July 14, 1998

ABSTRACT **Determinative events in vertebrate embryogenesis appear to require the continuous expression of spatial regulators such as the clustered homeobox genes. The mechanisms that govern long-term patterns of gene expression are not well understood. In *Drosophila*, active and silent states of developmentally regulated loci are maintained by *trithorax* and *Polycomb* group. We have examined the developmental role of a mammalian homolog of *trx* and putative oncogene, *Mll*. Knockout mice reveal that *Mll* is required for maintenance of gene expression early in embryogenesis. Downstream targets of *Mll* including *Hoxa7* are activated appropriately in the absence of *Mll* but require *Mll* for sustaining their expression. The *Mll*−/− phenotype manifests later in development and is characterized by branchial arch dysplasia and aberrant segmental boundaries of spinal ganglia and somites. Thus, *Mll* represents an essential mechanism of transcriptional maintenance in mammalian development, which functions in multiple morphogenetic processes.**

During vertebrate development, regions of the embryo become committed to position-specific identities (1, 2). For example, primordial thoracic segments retain their positional identity and form ribs even when ectopically placed into developing cervical regions (3). Moreover, genes that regulate spatial identities such as the evolutionarily conserved homeobox (*Hox*) genes display committed patterns of expression and are autonomously maintained (4). Studies of *Hox* gene regulation reveal dual phases of control: an early phase, which establishes the initial anteroposterior pattern of *Hox* expression, and a late phase, which is required for sustaining this pattern over successive cell generations (5–8). The maintenance of stable patterns of *Hox* expression may reflect more general mechanisms of gene regulation. In *Drosophila*, epigenetic mechanisms contribute to the long-term control of gene expression. *Trithorax* group (*trxG*) and *Polycomb*-group (*PcG*) maintain expression or repression of *Antennapediea* and *bithorax* homeotic gene complexes (9, 10) as well as other patterning genes including *engrailed* (11, 12) and *forkhead* (13). *trxG* and *PcG* proteins are found in association with chromatin and within large multimeric complexes; however, their mechanism of action is not completely understood (14).

Mammalian homologs of *trx* and *PcG* genes also have been identified and have been shown to regulate *Hox* expression (15, 16). *Mll* (also known as *Hrx*/*All1*) resembles *trx* and was isolated originally as a common target of chromosomal translocations in human acute leukemias (17, 18). *Mll* and *trx* encode large nuclear proteins with 9–10 zinc-finger motifs and a highly conserved 200 amino acid SET domain located at their carboxyl-terminal ends. *Mll* is widely expressed during embryogenesis. Gene-targeting studies in mice demonstrated that *Mll* is a positive regulator of *Hox* genes (19). *Hox* expression is shifted posteriorly in *Mll* heterozygous (+/−) embryos and completely abolished in *Mll* homozygous null (−/−) embryos. Shifts in *Hox* expression also are observed in mice with targeted mutations in *PcG* (20–23). Collectively, these findings indicate that, at least in part, *trxG* and *PcG* functions are conserved in mammals. Curiously, spatial patterns of *Hox* expression in *PcG* mutant and *Mll* mutant embryos once established remain stable over multiple embryonic stages. Whether *trxG*/*PcG* acts as maintenance factors in vertebrate development is not known. In this study, we determine that *Mll* affects gene maintenance rather than activation and explore the timing and biological relevance of maintenance mechanisms in mammalian development.

METHODS

Generation and Genotyping of *Mll*−/− Embryos. Knockout mice carrying an insertion of *lacZ* in the *Mll* gene were described (19). *Mll*+/− females were hypofertile but could be effectively bred between 8 and 14 weeks of age with proven *Mll*+/− males. Vaginal plugs were counted as embryonic day 0.5 (E0.5) of gestation (24, 25). Genomic DNA was prepared from yolk sacs of E9 and younger embryos and from aminotic sacs of older embryos as described (26, 27). Genotyping of embryos was performed by PCR by using a common upstream primer ST2B+ and two downstream primers, ST2B- (wild-type allele) and ST2L- (recombinant allele). Cycle conditions were as follows: 94°C × 5 min; 30 cycles of 94°C × 1 min, 60°C × 1 min, 72°C × 1 min; 72°C × 5 min. This PCR produced an 840-bp wild-type allele and a 400-bp recombinant allele. Primers used include ST2B+ (5′-GAACAGCAGATTCAGCGCCACG-3′), ST2B- (5′-GGACGCTCCAGAAGAAGTTCGATTA-3′), and ST2L- (5′-GAACAAACGGCGGATTGACCGTAATG-3′). *Mll-lacZ*+/− animals were identified from positive tail biopsies stained overnight in a X-Gal-staining solution (1.65 mg/ml $K_4Fe(CN)_6{\cdot}3H_2O$/2.1 mg/ml $K_3Fe(CN)_6$/2 mM $MgCl_2$/0.1% sodium deoxycholate/0.1% Nonidet-40/0.8 mg/ml X-Gal in PBS).

Histology and *in Situ* Cell Death Assay. Embryos prepared for histology were fixed in either newly prepared 4% paraformaldehyde in PBS or in 10% formalin and embedded in paraffin. Serial sections (5–7 μm) were stained with hematoxylin and eosin. To examine programmed cell death *in situ*, tissues were fixed in formalin and embedded in paraffin. The terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling method (28) (TUNEL) was used on 5- to 7-μm sections of 2–3 embryos of each genotype and performed according to manufacturer's methods (TdT TUNEL assay kit, Trevigen, Gaithersburg, MD). Briefly, sections were treated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: *trxG*, *trithorax*-group; *PcG*, *Polycomb*-group; *En1*, *Engrailed-1*; *Hox*, homeobox; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling; E0.5–E10.5, embryonic day 0.5–10.5.

*To whom reprint requests should be addressed. e-mail: skorsmey@imgate.wustl.edu.

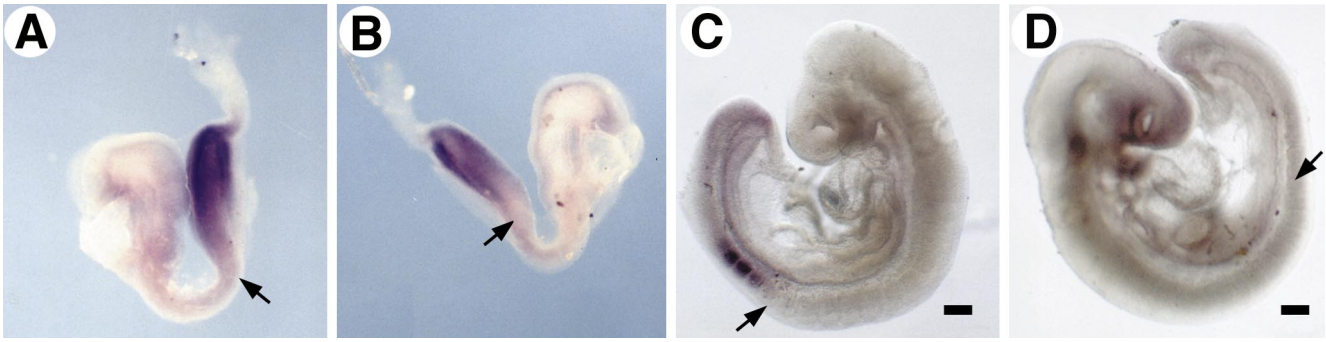

FIG. 1. Defective maintenance of *Hoxa7* expression in *Mll*−/− embryos. Whole mount *in situ* hybridization of E8.5 and E9 control and *Mll*−/− embryos (lateral views). (*A*) *Hoxa7* expression in the presomitic mesoderm and neural plate of E8.5 wild-type embryos. (*B*) *Hox-7* expression in E8.5 *Mll* knockout embryos. Arrows indicate the anterior most extent of *Hoxa7* expression. (*C*) *Hoxa7* expression in somites 15–18 and presomitic mesoderm in E9 *Mll*+/− embryos. (*D*) Absence of *Hoxa7* expression in somites of E9 *Mll*−/− embryos. Faint expression was detected in *Mll*−/− embryos proximal to the allantois. Arrows point to the anterior boundary of somite 15 (prospective eleventh vertebrae).

with proteinase K and hydrogen peroxide before incubation in terminal deoxynucleotidyltransferase and biotin-labeled nucleotides. Apoptotic cells then could be detected by staining with a strepavidin-horseradish peroxidase conjugate.

***In Situ* Hybridization and Immunohistochemistry.** *In situ* hybridizations on sections were performed with ^{33}P-labeled (Amersham) riboprobes whereas whole mount *in situ* hybridizations were stained with digoxygenin-labeled riboprobes according to manufacturer's protocols (Boehringer Mannheim). Details of *in situ* hybridization protocols are described elsewhere (29). Riboprobes were generated from *Hoxa7* (363 bp *Sma*I), *Hoxc8*, *Pax1*, and *myf5* DNA templates, and both sense and anti-sense orientations were tested on wild-type embryos. For whole mount neurofilament staining, embryos

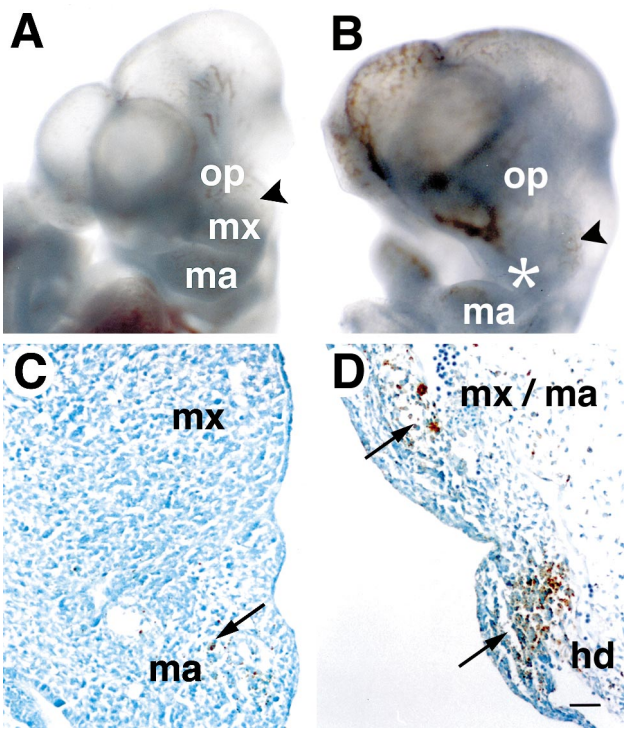

FIG. 2. Branchial arch abnormalities in *Mll* knockout embryos. Gross views and TUNEL staining of control and *Mll*−/− E10.5 embryos. (*A*) Wild-type embryo (oblique view), demonstrating normal structures of the first branchial arch, maxillary prominence (MX), the mandibular component (MA), and associated trigeminal ganglia (arrowhead). (*B*) *Mll*−/− embryo revealing branchial arch hypoplasia and defects in the development of the maxilla (asterisk). (*C*) First branchial arch of *Mll*+/+ embryos. Apoptotic cells are indicated by long arrows. (*D*) First and second branchial arches of *Mll*−/− embryos; same magnification as (*C*). Note the severe hypocellularity and apoptosis in the arch mesenchyme but not the surface ectoderm. op; optic vesicle; HD, hyoid or second branchial arch. (Scale bar = 50 μm.)

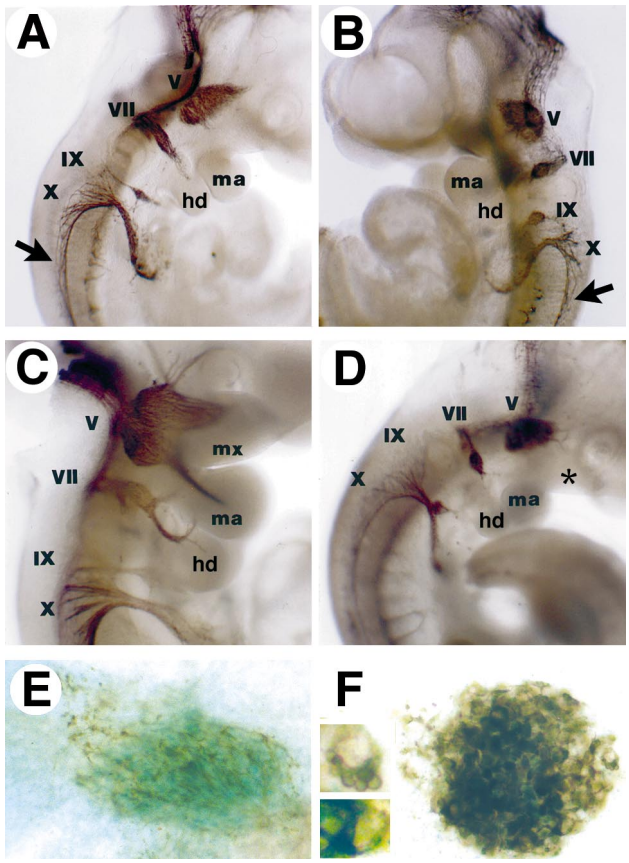

FIG. 3. Cranial ganglia development in wild-type and mutant embryos at E10 and E10.5. Neurofilament immunostaining of whole embryos and isolated facial ganglia (lateral views). (*A*) E10 wild-type embryo demonstrating normal morphology of cranial ganglia. (*B*) E10 *Mll*−/− embryo displaying condensed morphology of ganglia and present branchial arch structures. Spinal accessory nerves are indicated by arrows. (*C*) E10.5 *Mll*+/− embryo showing cranial innervation of the branchial arches. (*D*) E10.5 *Mll*−/− cranial ganglia are condensed and innervation of the branchial arches is absent. Defective positioning of the nodose ganglia was noted in only one of six knockout embryos. Note hypoplastic branchial arch development (*). (*E*) Flat mount of *Mll*+/− facial ganglia; blue staining reflects expression of *Mll-lacZ* marker in cranial ganglia. (*F*) *Mll*−/− facial ganglia. (*Inset*) Magnified view of cells found within the facial ganglia. V, trigeminal; VII, facial; IX, glossopharyngeal; X, vagal nerves.

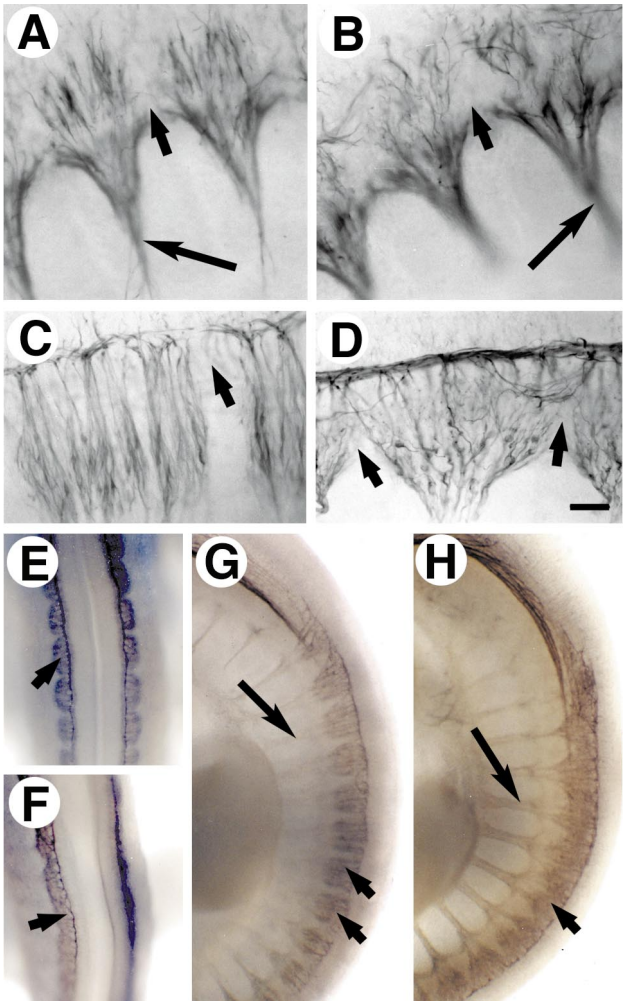

FIG. 4. Defective neurite outgrowth and segmentation of *Mll*−/− spinal ganglia. Immunohistochemical staining for neurofilament was performed on E10 and E10.5 embryos. (*A*) Flat mount of E10 *Mll*+/+ spinal ganglia and motor nerves (long arrow). Short arrows indicate boundaries between somites. (*B*) E10 *Mll*−/− spinal ganglia and motor nerves from similar axial level as shown in *A*. Neurites of knockout spinal ganglia are disorganized but did not cross somite boundaries. (*C*) E10.5 wild-type spinal ganglia. (*D*) E10.5 *Mll*−/− spinal ganglia demonstrating aberrant morphology and loss of segmental restriction. (*E*) Dorsal view of segmented E10.5 *Mll*+/+ spinal ganglia at the forelimb level. Short arrows indicate spinal ganglia. (*F*) Fused E10.5 *Mll*−/− spinal ganglia. (*G*) Lateral view of segmented spinal ganglia and spinal nerves in E10.5 *Mll*+/− embryo. (*H*) E10.5 *Mll*−/− embryo displays spinal ganglia fusion. Fusion of spinal ganglia was pronounced less posteriorly. Note the segmented pattern of spinal and motor nerves.

were fixed in 4% paraformaldehyde in PBS, stained with 2H3 mouse mAb and peroxidase-conjugated rabbit secondary antibody (Sigma), and developed with 4-chloro-1-naphtol (30). *En1* and *En2* staining were performed at 1:100 dilution of αEnhb-1 rabbit antibody as described (31). Stained trunks were bisected, cleared in glycerol, and mounted on coverslips. Whole embryos were photographed on agarose plates by using an Olympus SZH bifocal dissecting scope (New Hyde Park, NY). Sections and flat-mounted tissue were scanned by the Leaf Micro Lumina camera system (Zeiss) mounted on a Zeiss Axiophot microscope. Images were captured on an Apple Macintosh 7500 by using ADOBE PHOTOSHOP 3.0. *In situ* hybridization experiments were photographed under dark field optics. For all studies described here, three or more embryos of each genotype and embryonic stage were studied with the exception of *in situ* hybridization of sections. Serial

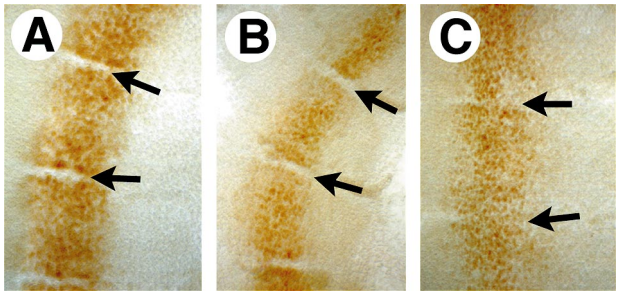

FIG. 5. Distribution of *EN1*-positive cells in E10.5 somites of wild-type and mutant embryos (lateral views). Flat-mounted somites immunostained with *EN1/2* antibody. (*A*) *EN1* expression in anterior somites of wild-type embryos; *EN1* expression at this stage was in the dermomyotome. Somites shown are at the level of the forelimb. Arrows indicate the somite boundaries. (*B*) *EN1* in posterior somites of *Mll*−/− embryo. (*C*) *EN1* expression in anterior somites of *Mll*−/− embryos taken at the level of the forelimb. Note the presence of *EN1* positive cells between somites.

sections of two embryos of each genotype were used for the analysis of *Pax1* and *myf5* expression.

RESULTS

Mll regulates *Hoxa7*, *Hoxc9*, and other *Hox* genes. *Hoxa7* regulation undergoes activation between E7.5 and E8.5 followed by a later stage of tissue-specific maintenance (7). To investigate whether *Mll* plays a role in gene activation or maintenance, *Hoxa7* expression was examined in *Mll*−/− embryos from E8 and time points thereafter. Before E9, the pattern and level of *Hoxa7* expression were similar between wild-type (Fig. 1*A*) and *Mll*−/− embryos (Fig. 1*B*). Expression of *Hoxa7* was seen in the neuroectoderm and the presomitic mesoderm of E8.5 embryos. At E9, somitic expression of *Hoxa7* in wild-type (not shown) and heterozygous embryos (Fig. 1*C*) demonstrated establishment of the anterior boundary of *Hoxa7*. *Hoxa7* expression was relatively weak in the neural tubes of wild-type embryos at this stage. In contrast, *Mll*−/− embryos showed no substantial expression of *Hoxa7* in the somites or neural tube at E9, and only faint staining could be detected in the presomitic mesenchyme (Fig. 1*D*). *Hoxc8*, which is regulated by early and late response elements (8), also failed to maintain expression beyond E9 (not shown). Gene expression was not globally suppressed in *Mll*−/− embryos as determined by *in situ* hybridization of somite lineage markers (see below). Thus, the loss of late *Hox* expression in the absence of *Mll* indicates that *Mll* functions as a maintenance factor.

Persistent expression of spatial regulators has been implicated in the control of committed identities of tissues such as neural crest and somites (1, 2). To determine the fate of cranial neural crest in the absence of *Mll*, we examined branchial arch development and cranial ganglia patterning. *Mll* knockout mice were embryonic lethal at E10.5 and displayed hypoplasia of all branchial arches and involution of the maxillary process (Fig. 2 *A* and *B*). The mesenchyme of arch tissue was hypocellular and showed evidence of apoptosis as confirmed by TUNEL staining (Fig. 2 *C* and *D*). Neural crest cells, which contribute to the expansion and outgrowth of arch mesenchyme, also play an important role in the development of other craniofacial structures including cranial ganglia (32).

Whole mount immunohistochemical staining for neurofilament revealed that early cranial ganglia development, migration, and differentiation proceeded normally in the absence of *Mll*. However, slight changes in cranial gangliogenesis became apparent as early as E10 (Fig. 3 *A* and *B*). Facial and nodose ganglia of *Mll* knockout embryos consistently displayed a compact, globoid morphology, which was not representative of

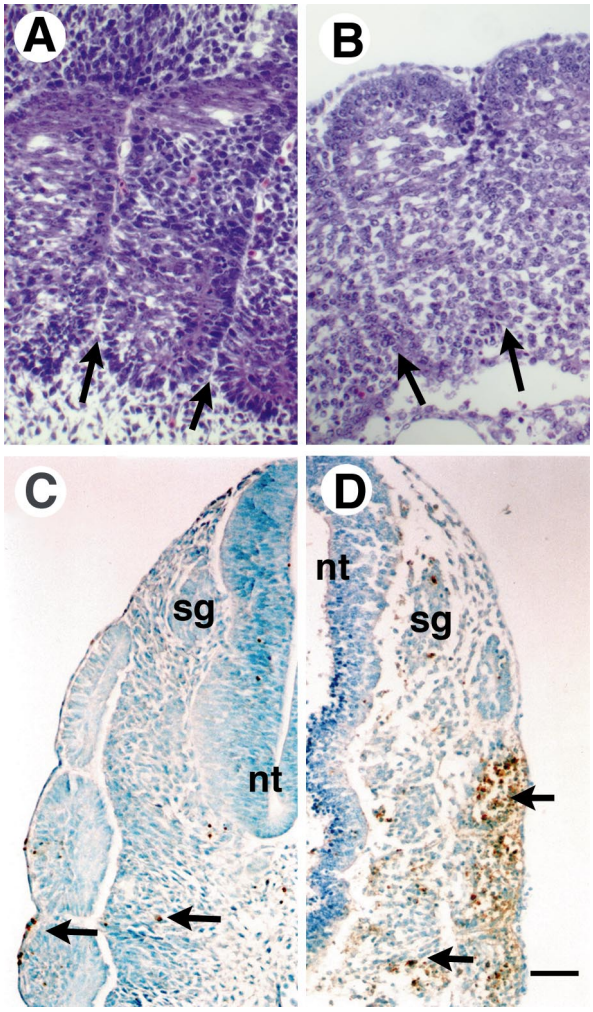

FIG. 6. Somite architecture and cell death in wild-type and *Mll*−/− E10.5 embryos. Hematoxylin and eosin staining of lateral sections and TUNEL staining of transverse sections. (*A*) wild-type somites; note normal morphology of somite epithelium indicated by arrows. (*B*) *Mll*−/− somites demonstrating disrupted epithelial boundaries ventrally. (*C*) Normal cell death in wild-type somites revealed by TUNEL. (*D*) Cell death in *Mll*−/− somites; note the relative sparing of spinal ganglia (sg) and neural tube (nt). Arrows indicate areas of apoptosis. (Scale bars = 50 μm.)

any stage of normal development. By E10.5, whereas *Mll* wild-type and heterozygous embryos showed prominent nerve tracts innervating the arches, *Mll*−/− cranial ganglia continued to retain this unusual morphology and failed to elaborate nerve fibers toward branchial arch targets (Fig. 3 *C* and *D*) Flat-mounted facial ganglia revealed abundant neurofilament but were defective in neurite outgrowth (Fig. 3 *E* and *F*). Branchial arch tissues have a chemotropic role in cranial ganglia development (33), and thus defects in the cranial nerves may reflect underlying problems in the branchial arches. In support of this thesis, vagal and spinal accessory nerves, which have targets outside the branchial arches, were relatively spared in the *Mll*−/− embryos.

Neural patterning in the trunk also was affected by the loss of *Mll*. *Mll*−/− spinal ganglia showed disorganized neurite outgrowth as early as E10 (Fig. 4 *A* and *B*) and progressive loss of segmental boundaries through E10.5 (Fig. 4 *C* and *D*). Neurite projections from spinal ganglia crossed into neighboring rostral and caudal segments, and spinal ganglia became extensively fused in the *Mll*−/− E10.5 embryos (Fig. 4*E–H*). The anterior spinal ganglia were affected more severely than the posterior spinal ganglia suggestive of defect in the later

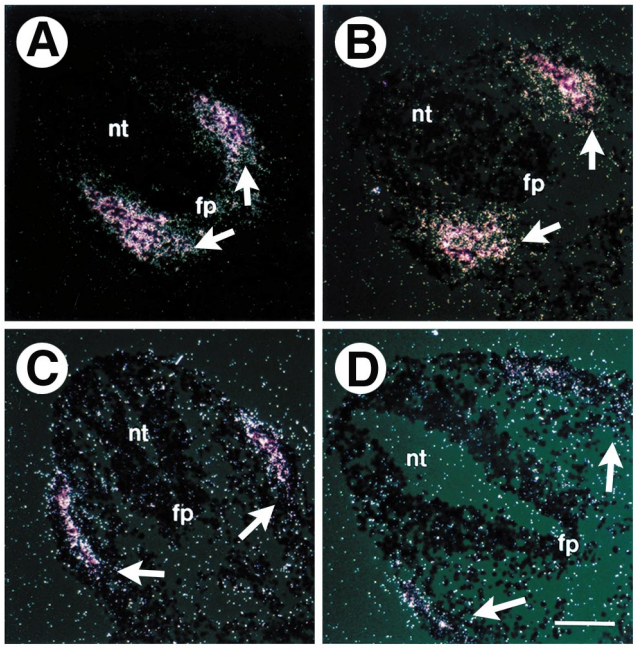

FIG. 7. Differentiation of somites in wild-type and *Mll*−/− E10.5 embryos. *In situ* hybridization of *Pax-1* (*A* and *B*) and *myf-5* (*C* and *D*) performed on transverse sections. (*A*) *Pax-1* expression in the sclerotome of wild-type embryo. White arrows show areas of expression. (*B*) *Pax-1* expression in *Mll*−/− embryo. (*C*) *Myf-5* expression in the dermomyotome of *Mll*+/+ embryo. (*D*) *Myf-5* expression in *Mll*−/− embryo. nt, neural tube; fp, floor plate. (Scale bars = 50 μm.)

stages of sensory nerve patterning. Motor nerves whose development precedes that of spinal ganglia showed a normal segmented distribution in the absence of *Mll* (Fig. 4 *A* and *B*).

The segmental pattern of spinal ganglia is determined by neighboring somites during trunk neural crest migration and neurite outgrowth (34, 35). To explore somite boundaries, we examined the distribution of *Engrailed-1* (*En1*), a segmentally expressed homeobox gene. Sharp boundaries of *En1* expression normally are present in both the newly formed posterior somites and differentiated anterior somites throughout the trunk (Fig. 5*A*) (31). Although *Mll*−/− embryos retained a normal segmental distribution of *En1* expression posteriorly (Fig. 5*B*), they failed to maintain sharp boundaries of *En1* expression anteriorly (Fig. 5*C*). This may reflect a loss of segmental boundaries between differentiated somites of the *Mll*−/− embryos rather than a regulatory defect in *En1* because *En1* and *En2* expression was not altered elsewhere in the embryo (not shown).

Histologic analysis of somitic tissue revealed disrupted somite architecture (Fig. 6 *A* and *B*). Extensive cell death was observed in somites of *Mll*−/− embryos as detected by TUNEL whereas only rare apoptotic cells were observed in wild-type and heterozygous embryos (Fig. 6 *C* and *D*). In contrast, *Mll*−/− spinal ganglia and spinal cord at E10.5 did not show significantly increased cell death.

Somites differentiate into sclerotome and dermomyotome; the sclerotome of differentiated somites has been shown to provide segmental signals to migrating trunk neural crest and axons of spinal ganglia (2). Somite maturation as assessed by expression of the sclerotome and dermomyotome lineage markers, *Pax1* (Fig. 7 *A* and *B*) and *myf5* (Fig. 7 *C* and *D*), respectively, was not appreciably altered in the *Mll*−/− embryos. These results argue that during differentiation, *Mll* is essential for the maintenance of segmental boundaries in somites and spinal ganglia.

DISCUSSION

In this study, we show that *Mll* acts as a maintenance factor necessary for correct development of multiple tissues during

embryogenesis. *Mll* is ubiquitously expressed and is required for successful skeletal (19), hematopoietic (27), neural, and craniofacial development. Maintenance mechanisms have been proposed to impart stable gene expression important for retaining cell identity and positional cues during cell proliferation, migration, and differentiation. The complex phenotype of the *Mll*−/− embryos could reflect the lack of sustained expression of spatial regulators. For example, the effects of *Mll* deficiency on branchial arch development and cranial nerve outgrowth are reminiscent of defects described in *Hox* knockout models (36). Progressive aberrations in the segmentation of somites and spinal ganglia also were found in *Mll* knockout embryos. Defective maintenance of genes involved in specifying the rostrocaudal polarity of somites might explain the later onset of segmentation defects in the *Mll*−/− embryos. The anteroposterior fate of *Mll*−/− somites also may be transformed but could not be accurately assessed because of early embryonic lethality. Segmentation defects in the somites might reflect the anterior transformation of mesoderm in the *Mll*−/− embryos. Interestingly, the anterior most mesoderm does not normally form discrete somites (6). The distribution of *Mll*−/−-derived cells in a chimeric mouse model would help to define their capacity to acquire different anteroposterior fates.

We have identified a critical interval between E8.5 and E9 after which gene expression becomes dependent on *Mll*. This stage in development may reflect a dynamic period of chromatin reorganization, which leads to either silencing or persistent expression of developmentally regulated loci. In the *Mll* knockout embryos, this balance may be influenced by loss of positive signals and/or by unopposed *PcG* proteins resulting in the silencing of *Hox* expression. The observed shifts in *Hox* patterns in *Mll*(+/−) and *PcG* mutant mice also may reflect defects incurred in this early period. During this critical window, patterns of *Hox* expression appear to stabilize set by the relative levels of *trxG*/*PcG* proteins. Interactions among *trx* and *PcG* proteins are thought to transmit active and silent chromatin states to successive cell generations thereby creating stable patterns of gene expression (37). Motifs within *Mll* such as the AT-hook and SET domain have been implicated in chromatin regulation (38, 39). Recently described interactions of the *Mll* SET domain with components of the SWI/SNF complex argue that *Mll* is involved in recruiting chromatin remodeling machinery and opening repressed loci (40, 41). The *Mll* knockout mice provide a model system for studying maintenance mechanisms involved in patterning the vertebrate body plan.

We thank Raphael Kopan for critical review of this manuscript. We thank P. Gruss for *Hoxa7* and *Pax1*; A. Schumacher for *Hoxc8*; J. Miner for *myf5*; A. Joyner for αEnhb-1 antibody; and Developmental Studies Hybridoma Bank for 2H3 neurofilament antibody. We thank Mary Pichler for expert secretarial assistance. This work was supported in part by a grant from The Parker Hughes Trust, B.D.Y. was supported by a training grant from the National Institutes of Health, and R.D.H. was supported by a physician fellowship from the Howard Hughes Medical Institute.

1. Noden, D. M. (1993) *J. Neurobiol.* **24,** 248–261.
2. Tam, P. P. L. & Trainor, P. A. (1994) *Anat. Embryol.* **189,** 275–305.
3. Kieny, M., Mauger, A. & Sengel, P. (1972) *Dev. Biol.* **28,** 142–161.
4. Beddington, R. S. P., Püschel, A. W. & Rashbass, P. (1992) in *Postimplantation Development in the Mouse*, eds. Chadwick, D. J. & Marsh, J. (Wiley, New York), pp. 61–77.
5. Deschamps, J. & Wijgerde, M. (1993) *Dev. Biol.* **156,** 473–480.
6. Gaunt, S. J. & Strachan, L. (1994) *Dev. Dyn.* **199,** 229–240.
7. Püschel, A. W., Balling, R. & Gruss, P. (1991) *Development (Cambridge, U.K.)* **112,** 279–287.
8. Bieberich, C. J., Utset, M. F., Awgulewitsch, A. & Ruddle, F. H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8462–8466.
9. Lewis, E. B. (1978) *Nature (London)* **276,** 565–570.
10. Sedkov, Y, Tillib, S., Mizrokhi, L. & Mazo, A. (1994) *Development (Cambridge, U.K.)* **120,** 1907–1917.
11. Chinwalla, V., Jane, E. P. & Harte, P. J. (1995) *EMBO J.* **14,** 2056–2065.
12. Moazed, D. & O'Farrell, P. H. (1992) *Development (Cambridge, U.K.)* **116,** 805–810.
13. Kuzin, B., Tillib, S., Sedkov, Y., Mizrokhi, L. & Mazo, A. (1994) *Genes Dev.* **8,** 2478–2490.
14. Pirrotta, V. (1998) *Cell* **93,** 333–336.
15. Gould, A. (1997) *Curr. Opin. Genet. Dev.* **7,** 488–494.
16. Schumacher, A. & Magnuson, T. (1997) *Trends Genet.* **13,** 167–170.
17. Tkachuk, D. C., Kohler, S. & Cleary, M. L. (1992) *Cell* **71,** 691–700.
18. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M. & Canaani, E. (1992) *Cell* **71,** 701–708.
19. Yu, B. D., Hess, J. L., Horning, S. E., Brown, G. A. J. & Korsmeyer, S. J. (1995) *Nature (London)* **378,** 505–508.
20. van der Lugt, N. M. T., Alkema, M., Berns, A. & Deschamps, J. (1996) *Mech. Dev.* **58,** 153–164.
21. Akasaka, T., Kanno, M., Balling, R., Mieza, M. A., Taniguchi, M. & Koseki, H. (1996) *Development (Cambridge, U.K.)* **122,** 1513–1522.
22. Coré, N. Bel, S., Gaunt, S. J., Aurrand-Lions, M., Pearce, J., Fisher, A. & Djabali, M. (1997) *Development (Cambridge, U.K.)* **121,** 2847–2852.
23. Takihara, Y., Tomotsune, D., Shirai, M., Katoh-Fukui, Y., Nishii, K., Motaleb, M. A., Nomura, M., Tsuchiya, R., Fujita, Y., Shibata, Y., *et al.* (1997) *Development (Cambridge, U.K.)* **124,** 3673–3682.
24. Hogan, B., Beddington, R., Constantini, F. & Lacy, E. (1994) in *Manipulating the Mouse Embryo* (Cold Spring Harbor Lab. Press, Plainview, NY).
25. Kaufman, M. H. (1992) in *The Atlas of Mouse Development* (Academic, San Diego).
26. Laird, P. W., Zijderveld, A., Linders, K., Rudnicki, M. A., Jaenisch, R. & Berns, A. (1991) *Nucleic Acids Res.* **19,** 4293.
27. Hess, J. L., Yu, B. D., Li, B., Hanson, R. & Korsmeyer, S. J. (1997) *Blood* **90,** 1799–1806.
28. Gavrieli, Y., Sherman Y. & Ben-Sasson, S. A. (1992) *J. Cell Biol.* **119,** 493–501.
29. Wilkinson, D. G. & Neito, M. A. (1993) *Methods Enzymol.* **225,** 361–373.
30. Swiatek, P. J. & Gridley, T. (1993) *Genes Dev.* **7,** 2071–2084.
31. Davis, C. A., Holmyard, D. P., Millen, K. J. & Joyner A. L. (1991) *Development (Cambridge, U.K.)* **111,** 287–298.
32. Serbedzija, G. N., Bronner-Fraser, M. & Fraser, S. E. (1992) *Development (Cambridge, U.K.)* **116,** 297–307.
33. Davies, A. M. (1988) *Development (Cambridge, U.K.)* **103,** Suppl., 175–183.
34. Kalcheim, C. & Teillet, M.-A. (1989) *Development (Cambridge, U.K.)* **106,** 85–93.
35. Keynes, R., Cook, G., Davies, J., Lumsden, A., Norris, W. & Stern, C. (1990) *J. Physiol. (Paris)* **84,** 27–32.
36. Gavalas, A. Studer, M., Lumsden, A., Rijli, F. M., Krumlauf, R. & Chambon, P. (1998) *Development (Cambridge, U.K.)* **125,** 1123–1136.
37. Paro, R. (1993) *Curr. Opin. Cell Biol.* **6,** 373–379.
38. Girard, F., Bello, B., Laemmli, U. K. & Gehring, W. J. (1998) *EMBO J.* **17,** 2079–2085.
39. Laible, G. (1997) *EMBO J.* **16,** 3219–3232.
40. Roszenblatt-Rosen, O., Rozovskaia, T., Burakov, D., Sedkov, Y., Tillib, S., Blechman, J., Nakamura, T., Croce, C. M., Mazo, A. & Canaani, E. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 4152–4157.
41. Kingston, R. E., Bunker, C. A. & Imbalzano, A. N. (1996) *Genes Dev.* **10,** 905–920.