females stain blue with a distinct red nucleus [P. C. C. Garnham, Malaria Parasites and Other Haemosporidia (Blackwell Scientific, Oxford, 1966)]. Sex ratios based on counts of 50 to 75 gametocytes were found to be representative. We calculated sex ratios from either 50,000 RBCs or 100 gametocytes, whichever was less. Sex ratios are given as proportion of males.


9. Infection classification by day with respect to peak parasitemia is retrospective, where P − 1, P − 2, and P − 3 are 1, 2, and 3 days before peak parasitemia. The proportions of sex and of type II DNA topoisomerase IIb (IIb) were estimated. The proportions of sex and of type II DNA topoisomerase IIb (IIb) were estimated. The proportions of sex and of type II DNA topoisomerase IIb (IIb) were estimated.

10. Statistical regression was performed in SIMSTAT for Windows (Provisal Research).

11. Two vaccination procedures were carried out according to protocols previously established (F. Hawking, K. Gammage, M. J. Worms, Trans. R. Soc. Liverpool 181, 199 (1972).

12. The first day parasites appeared in the blood (parasite titer) was infected with a single type II DNA topoisomerase, IIb.

13. Erythropoietic treatments were carried out on the first day of peak parasitemia and that information is supplied in the figure legends. Such variation was taken into account in the statistical analyses.

14. Erythropoietic treatments were carried out on the first day of peak parasitemia and that information is supplied in the figure legends. Such variation was taken into account in the statistical analyses.


19. Two vaccination procedures were carried out according to protocols previously established (F. Hawking, K. Gammage, M. J. Worms, Trans. R. Soc. Liverpool 181, 199 (1972).


IIβ detected the protein in extracts of fibroblasts from E13.5 WT but not top2β−/− embryos (7). Wild-type and top2β+/− embryos are comparable in size up to E15.5, but homozygous mutant embryos show retarded growth thereafter; at E18.5 the average weight of top2β−/− embryos is ~65% that of their WT littermates. The top2β−/− embryos also exhibit a curled appearance because of an abnormal curvature of their vertebral columns (Fig. 1B). Examination of the top2β−/− embryos showed no gross morphological abnormality in major organs. These embryos, however, lacked spontaneous and tactile-stimulated movements. Their lungs involuted remained collapsed after birth, indicating that a respiratory failure is the most likely cause of their perinatal death.

The failure of top2β−/− newborns to move or breathe suggested a defect in neuromuscular function. The number of motor neurons or interneurons in WT and mutant embryos at E12.5 appears to be similar, as revealed by staining spinal sections with appropriate antibodies. Furthermore, in E12.5 top2β−/− embryos, sensory as well as motor neurons extend their axons into the periphery; motor axons, specifically labeled by injecting DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) into the ventral spinal cord (8), project into the limbs by E12.5 and continue to grow further into the limbs by E13.5 [Web figure 2 (6)]. Thus, early aspects of motor neuron differentiation, including neurogenesis, appear normal in top2β−/− embryos.

We studied neuromuscular synapses in top2β−/− embryos by staining whole mounts of diaphragm muscles with probes that allowed us to assess presynaptic and postsynaptic differentiation (9); a mixture of antibodies to neurofilaments (NF) and a synaptic vesicle protein synaptophysin (Syn) was used to stain axons and nerve terminals, respectively, and α-bungarotoxin (α-BGT) was used to stain postsynaptic acetylcholine receptors (AChRs). In WT mice, motor axons branch and terminate adjacent to the main intramuscular nerve, resulting in a narrow, well-defined endplate zone in the middle of the diaphragm muscle (Fig. 2A). Presynaptic nerve terminals precisely overlie the postsynaptic membrane, which contains a high concentration or cluster of AChRs (Fig. 2A). In E18.5 top2β−/− embryos, presynaptic nerve terminals and axons are absent from the diaphragm muscle (Fig. 2A). Similar results were obtained by staining longitudinal sections of limb muscles from mutant embryos (7). We considered the possibility that early motor axons failed to reach their skeletal muscle targets or that these axons contacted muscle and subsequently withdrew. Staining of whole mounts of diaphragm muscles at E13.5, E15.5, and E18.5 revealed that motor axons in the mutant embryos extended toward the diaphragm muscle but failed to grow or branch within the muscle at all stages examined (Fig. 2B). In contrast, motor axons in WT embryos reached the diaphragm muscle by E13.5 and continued to grow within the muscle and to form a well-patterned endplate zone (Fig. 2B). These data indicate that motor axons in top2β−/− embryos reach their skeletal muscle targets but fail to grow or branch within their limb and diaphragm muscles and to contact differentiating muscle fibers.

The presence of primary nerve trunks but not secondary branches in the developing limbs of top2β−/− embryos is reminiscent of the motor axon pattern in muscle-less limbs (10), and points to a plausible defect in communication between muscle cells and motor axons in the mutant embryos. Because growth of nociceptive sensory neurons that innervate skin and not muscle is also defective in top2β−/− embryos (see below), we favor the idea that IIβ is required in neurons, rather than in muscles, for the expression of molecules essential for receiving cues for active-site tyrosine codon. The hatched bars mark the positions of probes used in genotyping; the “5’ probe” was prepared by polymerase chain reaction with primers represented by the arrows 1 and 2 [see Supplementary Web material (6) for details on the construction of the targeting vector and examples of genotyping and mRNA blot-hybridization results]. (B) Images of E17.5 WT (left) and top2β−/− (right) embryos.
dermomyotome (13), but only the precursors for diaphragm and limb muscles express the homeobox gene Lbx1 and migrate from the somite to the septum transversum or limb, respectively (44). Thus, the lack of top2β−/− may have different manifestations in muscles of different lineages.

Sensory axon growth, however, is aberrant in intercostal muscles. In WT embryos motor axons branch and terminate adjacent to the main intramuscular nerve, and sensory axons branch toward the rostral rib and terminate near muscle insertions on both sides of the rib (Fig. 3, A to C). NF-stained axons in top2β−/− embryos stray from the main nerve and grow profusely across the rib (Fig. 3A). By injecting DiI into the ventral horn of the spinal cord to selectively label motor axons (Fig. 3B), or into dorsal root ganglia to selectively label sensory axons (Fig. 3C), these ectopic axons in top2β−/− embryos are shown to be derived from sensory and not motor neurons.

Sensory neuron defects in top2β−/− embryos are not restricted to axon growth in skeletal muscle. Proprioceptive sensory neurons extend their primary axons into the ventral region of the spinal cord, where they terminate on interneurons or directly on motor neurons. In addition, these proprioceptive sensory neurons extend collaterals in the dorsal spinal cord, and these collateral axons form the dorsal column that terminates in the medulla. Top2β−/− embryos lack the dorsal column (Fig. 3D). Furthermore, axons of nociceptive sensory neurons that project to the dorsal horn of the spinal cord are absent in top2β−/− embryos (Fig. 3D). Because nociceptive sensory neurons have their peripheral endings in epidermis and not in skeletal muscle, the failure of these neurons to project within the spinal cord is unlikely to be owing to a requirement of the enzyme in skeletal muscles. Because these axons are absent from the spinal cord as early as E14.5 (7), the time of their normal projection into the spinal cord, sensory axons in top2β−/− embryos apparently fail to initiate growth within the spinal cord, rather than entering and withdrawing at later stages.

The pronounced neural and neuromuscular abnormalities in the top2β−/− embryos are observed at late stages of embryogenesis when neuron and muscle cells are well differentiated. Thus, these defects are unlikely to reflect a general replicative or transcriptional role of IIβ. Defects in neurogenesis, associated with an increase in apoptosis, are also evident in mice lacking XRCC4 or DNA ligase IV (15, 16). XRCC4 protein and DNA ligase IV are components in a DNA repair complex, and thus their effects on neurogenesis may re-
Defects in sensory projections within intercostal muscles and spinal cord of top2β−/− embryos. (A) Whole mounts of intercostal muscles from E18.5 embryos, stained with antibodies to NF, show that NF-stained axons grow aberrantly across intercostal muscles and ribs. M and R mark the muscle and rib regions, respectively. (B) Motor axons of E18.5 embryos, labeled by injecting DiI into the ventral lateral spinal cord (DiI-MN), project normally in intercostal muscles. (C) Sensory axons, labeled by injecting DiI into the dorsal root ganglia (DiI-DRG), project ectopically across the ribs (arrows). Fewer ectopic sensory axons are seen in top2β−/− embryos by DiI labeling (C) than by NF staining (A), owing to the low DiI-labeling efficiency of sensory neurons. (D) Cross sections of the spinal cord from E18.5 embryos stained with antibodies to p75, which stains sensory as well as motor neurons, show that the dorsal column (arrows) is absent in top2β−/− embryos. Nociceptive sensory axons, which grow and terminate within the dorsal horn of the spinal cord in WT embryos (arrowheads), are also absent in top2β−/− embryos.

References and Notes
21. We thank C. L. Li, A. McMahon, and E. Robertson for materials and advice, H. Warren for help in pathologic examinations of mice, and A. Sharpe and L. Du for performing blastocyst injections. The gifts of antibodies from T. Jessell and M. Chao are gratefully acknowledged. We also thank M. Maitre for advice on Dil labeling. Supported by a NIH postdoctoral fellowship to K.Y. (NS10537) and by NIH research grants to S.J.B. (NS27963 and NS36193) and to J.C.W. (CA47958 and GM24544).

Tbx5 and the Retinotectum Projection
Kazuko Koshiba-Takeuchi,1,* Jun K. Takeuchi,1* Ken Matsumoto,1 Tsuyoshi Momose,1 Kenichiro Uno,1 Veit Hoepker,2 Keiko Ogura,3 Naoki Takahashi,1 Harukazu Nakamura,3 Kunio Yasuda,1 Toshihiko Ogura1†

Dorsal and ventral aspects of the eye are distinct from the early stages of development. The developing eye cup grows dorsally, and the choroidal fissure is formed on its ventral side. Retinal axons from the dorsal and ventral retina project to the ventral and dorsal tectum, respectively. Misexpression of the Tbx5 gene induced dorsalization of the ventral side of the eye and altered projections of retinal ganglion cell axons. Thus, Tbx5 is involved in eye morphogenesis and is a topographic determinant of the visual projections between retina and tectum.

Dorsal (medial) and ventral (lateral) aspects of the eye are distinct from early stages of development, and retinal axons project in an organized topographic manner (1, 2). Chick Tbx5 gene, a member of the T-box transcription factor family, is expressed in the dorsal
DNA Topoisomerase IIβ and Neural Development
Xia Yang, Wei Li, Elizabeth D. Prescott, Steven J. Burden and James C. Wang (January 7, 2000)
Science 287 (5450), 131-134. [doi: 10.1126/science.287.5450.131]

Editor's Summary