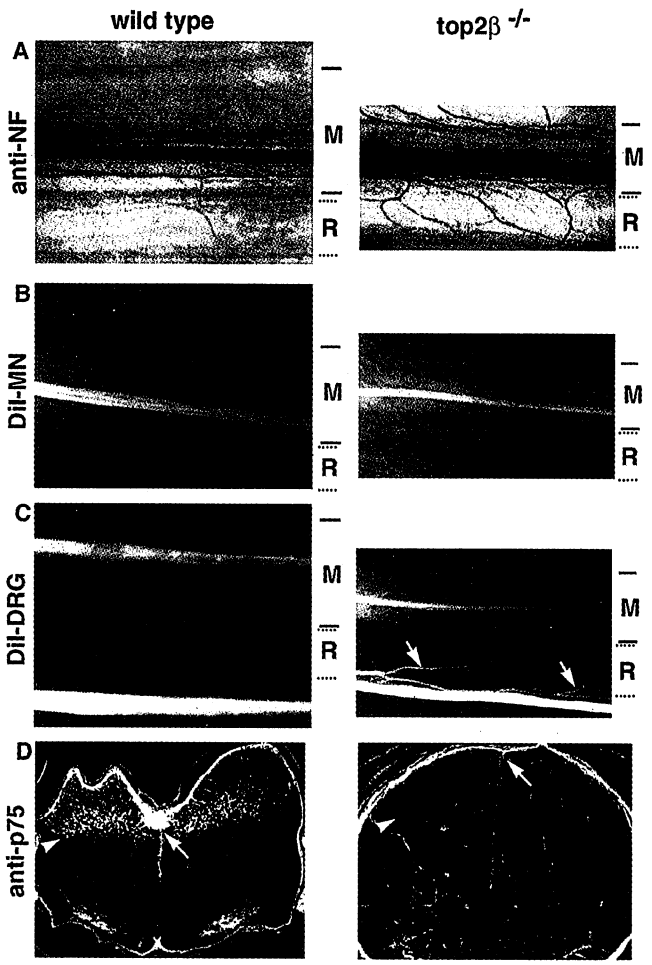

Fig. 3. Defects in sensory projections within intercostal muscles and spinal cord of *top2*β$^{-/-}$ embryos. (**A**) Whole mounts of intercostal muscles from E18.5 embryos, stained with antibodies to NF, show that NF-stained axons grow aberrantly across intercostal muscles and ribs. M and R mark the 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 *top2*β$^{-/-}$ 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 *top2*β$^{-/-}$ embryos. Nociceptive sensory axons, which grow and terminate within the dorsal horn of the spinal cord in WT embryos (arrowheads), are also absent in *top2*β$^{-/-}$ embryos.

flect a repair deficiency in their absence. It has been suggested that neurons may be more sensitive to repair deficiency than other cell types (*15*). Thus, the observed neural defects in IIβ mutant embryos might be related to the plausible involvement of IIβ in DNA repair (*17*). In budding yeast, DNA topoisomerases I and II suppress mitotic recombination in the ribosomal RNA gene cluster (*18*), and inactivation of IIβ could accentuate genome instability in neurons. The possible involvement of IIβ in gene expression, especially in nonproliferating cells like neurons that express no IIα, also deserves consideration in view of recent findings that eukaryotic type II DNA topoisomerase can form complexes with proteins implicated in gene expression (*19*) and chromatin remodeling (*20*).

References and Notes

1. Reviewed in C. A. Austin and K. L. Marsh, *Bioessays* **20**, 215 (1998); J. C. Wang, *Annu. Rev. Biochem.* **65**, 635 (1996).
2. S. DiNardo, K. Voelkel, R. Sternglanz, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2616 (1984); C. Holm, T. Goto, J. C. Wang, D. Botstein, *Cell* **41**, 553 (1985); T. Uemura *et al.*, *Cell* **50**, 917 (1987).
3. Reviewed in M. Yanagida and R. Sternglanz, in *DNA Topology and Its Biological Effects*, N. R. Cozzarelli and J. C. Wang, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990), pp. 299–320.
4. M. Chen and W. T. Beck, *Oncol. Res.* **7**, 103 (1995); S. Dereuddre, S. Frey, C. Delaporte, A. Jacquemin-Sablon, *Cancer Res.* **57**, 4301 (1997); P. Grue *et al.*, *J. Biol. Chem.* **273**, 33660 (1998).
5. B. Hogan, R. Beddington, F. Constatini, E. Lacy, *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994)
6. Supplemental Web material is available at www.sciencemag.org/feature/data/1045398.shl.
7. X. Yang, W. Li, E. D. Prescott, S. J. Burden, J. C. Wang, data not shown.
8. P. Godement, J. Vanselow, S. Thanos, F. Bonhoeffer, *Development* **101**, 697 (1987); M. G. Honig and R. I. Hume, *Trends Neurosci.* **12**, 333 (1989).
9. T. M. DeChiara *et al.*, *Cell* **85**, 501 (1996).
10. J. Lewis, A. Chevallier, M. Kieny, L. Wolpert, *J. Embryol. Exp. Morphol.* **64**, 211 (1981); B. Kablar and M. A. Rudnicki, *Dev. Biol.* **208**, 93 (1999).
11. J. R. Sanes and J. W. Lichtman, *Annu. Rev. Neurosci.* **22**, 389 (1999).
12. P. E. Williams and G. Goldspink, *J. Cell Sci.* **9**, 751 (1971).
13. C. P. Ordahl and N. Le Douarin, *Development* **114**, 339 (1992); C. P. Ordahl and B. A. Williams, *BioEssay* **20**, 357 (1998); reviewed in B. Christ and C. P. Ordahl, *Anat. Embryol.* **191**, 381 (1995).
14. S. Dietrich, F. R. Schubert, C. Healy, P. T. Sharpe, A. Lumsden, *Development* **125**, 2235 (1998).
15. Y. Gao *et al.*, *Cell* **95**, 891 (1998); D. E. Barnes, G. Stamp, I. Rosewell, A. Denzel, T. Lindahl, *Curr. Biol.* **8**, 1395 (1998).
16. K. M. Frank *et al.*, *Nature* **396**, 173 (1998).
17. O. Popanda and H. W. Theilmann, *Carcinogenesis* **13**, 2321 (1992); H. W. Thielmann, O. Propanda, H. Gersbach, F. Gilberg, *Carcinogenesis* **14**, 2341 (1993); K. Yamane, M. Kawabata, T. Tsuruo, *Eur. J. Biochem.* **250**, 794 (1997).
18. M. F. Christman, F. S. Dietrich, G. R. Fink, *Cell* **55**, 413 (1988); R. A. Kim and J. C. Wang, *Cell* **57**, 975 (1989).
19. M. Kobayashi *et al.*, *Mol. Cell. Biol.* **18**, 6737 (1998).
20. P. D. Varga-Weisz *et al.*, *Nature* **388**, 598 (1997).

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Tbx5 and the Retinotectum Projection

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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

side of the developing eye (*3*) and is homologous to the *Drosophila optomotor blind* (*omb*) gene, which regulates optic lobe development (*4*). Therefore, the Tbx5 gene could be a good candidate for a determinant of the dorsal-ventral (D-V) axis of the eye.

Tbx5 expression in the chick eye was first detected at stage 11 throughout the retina, with the strongest signal in the dorsal retina (Fig. 1A). Expression was later confined to the dorsal eye cup (Fig. 1, B and C) (*5*). *Tbx5* is expressed in both retinal pigment epithelium (RPE) and neural retina (NR) at these early stages. Although expression in RPE started to fade out at embryonic day (E) 10, robust expression was maintained in the dorsal NR (all layers except the nerve fiber layer at the inner surface of the retina; Fig. 1, D to F).

To analyze the function of Tbx5 in D-V axis determination, we used in ovo electroporation to express the *Tbx5* gene in the ventral eye (*6*, *7*). This method allows temporary site-directed expression of foreign genes. We used plasmid CAGGS-Tbx5-EGFP, which expresses Tbx5 under the control of the cytomegalovirus-β actin hybrid promoter (*8*). Expression of the transgene can be monitored by green fluorescent protein (GFP) fluorescence derived from Tbx5-EGFP fusion protein. When this plasmid was electroporated to the eye vesicles of stage 8 chick embryos, oval-shaped eyes were formed, presumably because of elongation of the eye cup in both dorsal and ventral directions (Fig. 1H). In these eyes, the location of the lens was shifted dorsally.

Pax2 is involved in the formation of the ventral structures (*9*). When CAGGS-Tbx5-EGFP was electroporated into the ventral half (Fig. 2D), expression of *Pax2* was repressed (Fig. 2E). Chicken *Vax* gene expressed in the ventral side of the developing eye cup (*10*) was also repressed (Fig. 2B). In addition, ventrally expressed *EphB2* and *EphB3* were also repressed (*11*). In contrast, the *EphrinB1* and *EphrinB2* genes were expressed only in the dorsal side (Fig. 2, I and K). When *Tbx5* was misexpressed, both *EphrinB1* and *EphrinB2* genes were induced in the ventral retina (Fig. 2, H and J). Thus, misexpression of *Tbx5* in the ventral side of the eye results in repression of the ventral markers *Pax2*, *Vax*, *EphB2*, and *EphB3* and induction of the dorsal markers *EphrinB1* and *EphrinB2* with

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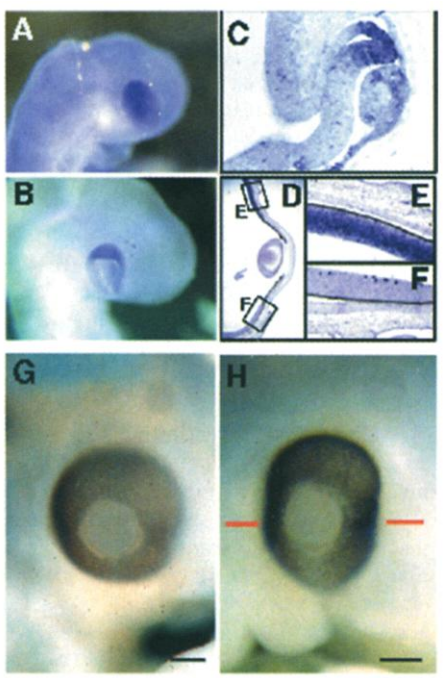

Fig. 1. Expression patterns of *Tbx5* gene in the developing eye. (**A**) Expression of *Tbx5* gene is detected in a broad area at stage 11. (**B**) At stage 14, expression becomes restricted to the dorsal half. (**C**) *Tbx5* is expressed in a graded fashion with the strongest signal at the dorsal-most end. (**D** to **F**) At E10, dorsal expression is maintained only in the NR. *Tbx5* transcripts were never detected in the ventral retina except for a few cells at the inner surface of the NR (F). (**G** and **H**) Misexpression of the *Tbx5* gene in the ventral side induces dorsalization of the ventral eye. This eye extends both dorsally and ventrally (H). The lens was shifted dorsally in this oval-shaped eye, whereas the normal eye is round and the lens is located ventrally (G). Red lines indicate the midline of the oval-shaped eye (H). Bars in (G) and (H), 1 mm.

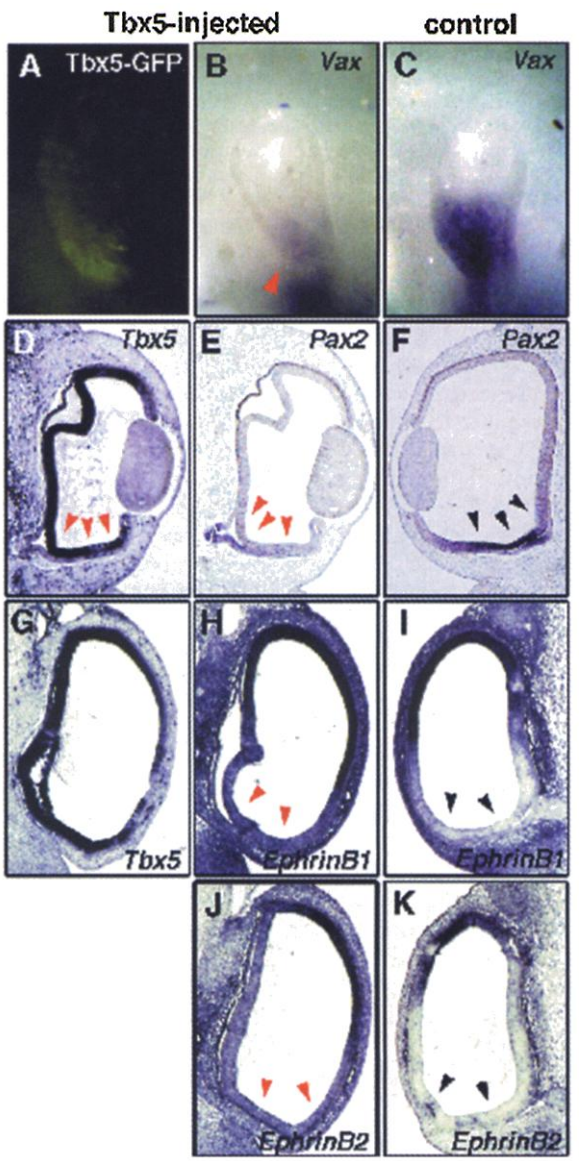

Fig. 2. Misexpression of *Tbx5* gene represses ventral markers *Pax2* and *Vax* and induces dorsal markers *EphrinB1* and *EphrinB2*. Misexpression was monitored by GFP signals derived from the *Tbx5-EGFP* fusion gene (**A**) and the section hybridization (**D** and **G**). (**B**) *Vax* expression was repressed by misexpression of Tbx5 (an arrowhead), whereas the control eye shows the robust expression in the ventral half (**C**). (**E**) *Pax2* was also repressed, whereas the control eye shows the normal expression (**F**). (**H** and **J**) Tbx5 induces dorsal markers *EphrinB1* and *EphrinB2*, which are not expressed in the ventral side of the normal eye cup (**I** and **K**).

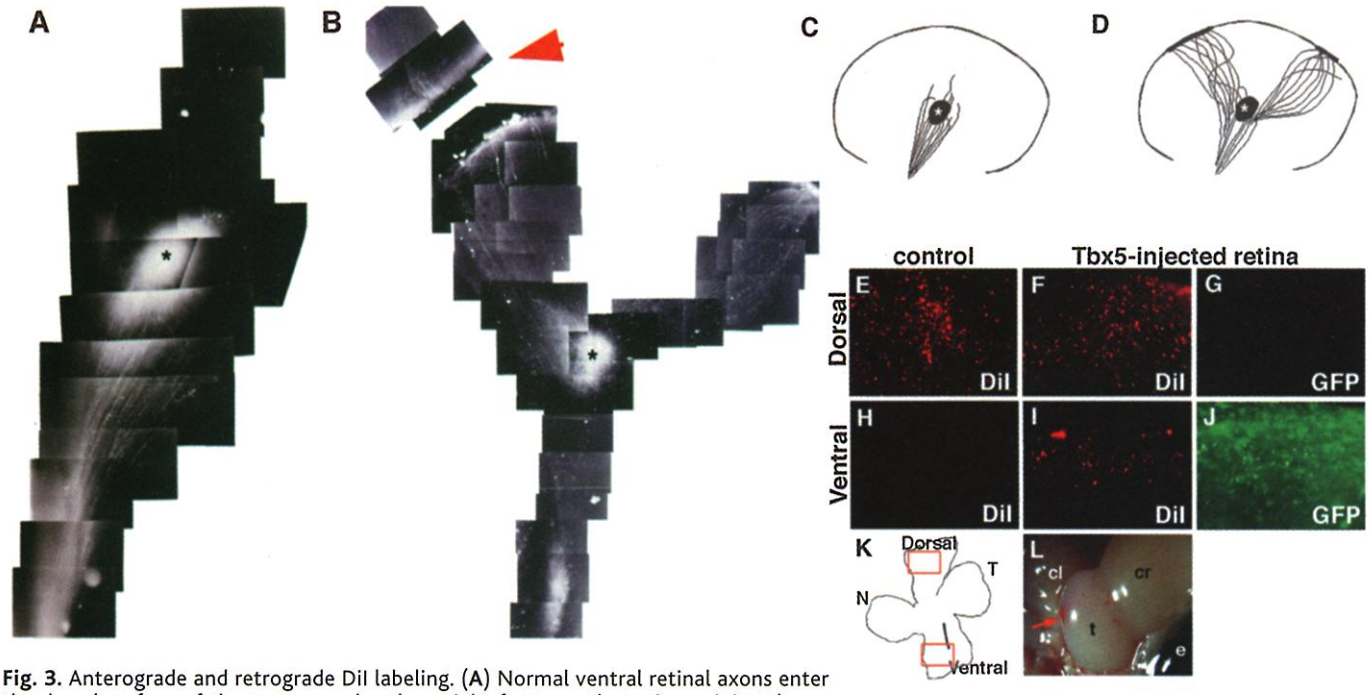

Fig. 3. Anterograde and retrograde DiI labeling. (**A**) Normal ventral retinal axons enter the dorsal surface of the tectum and make a tight focus on the retinorecipient layer, indicated by an asterisk. (**B**) When Tbx5 was misexpressed, ventral retinal axons did not converge on an appropriate spot (indicated by an asterisk). Instead, they passed it and converged to two aberrant spots with abnormal arborization and synaptic varicosities. Some axons were found in the opposite dorsal side of the tectum (red arrowhead). (**C** and **D**) Schematic drawings of (A) and (B), respectively. Caudal to the left, rostral to the right. (**E** to **J**) Retrograde DiI labeling. In normal retina, DiI signals were found exclusively in the dorsal half (E) and not in the ventral half (H). When Tbx5-EGFP was misexpressed in ventral retina, GFP signals were observed in the ventral retina (J) and not in the dorsal retina (G). In such retina, DiI signals were found in both dorsal (F) and ventral retina (I). (**K**) Schematic representation of the areas analyzed (small rectangles). N, nasal; T, temporal. (**L**) A spot made by DiI injection is shown (indicated by a red arrow). cr, cerebrum; cl, cerebellum; e, eye; t, tectum.

dorsalization of the ventral side of the eye.

Between E6 and E12, axons of retinal ganglion cells begin to enter the optic tectum and establish synaptic connections (*2*). To analyze possible involvement of *Tbx5* expression on the formation of retinotectum projections, we injected RCASBP-Tbx5-EGFP retrovirus into the optic cups at stage 8 to 10 (*12*) and then analyzed both retinas and tectums by anterograde DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) labeling (*13*, *14*). Virus injection was chosen because rapid transgene expression by electroporation induces dorsalization of the ventral eye, which might interfere with the coordinated exit of retinal axons into the optic stalk. Injection of retrovirus did not induce morphological changes, perhaps because there is a time lag (around 16 hours) between injection of virus and the full expression of genes (*15*).

Ventral retinal axons normally converge on a terminal zone in the retinorecipient layer of the dorsal side of the tectum (n = 15) (*2*) (Fig. 3A). In contrast, in 7 out of 30 embryos infected with RCASBP-Tbx5-EGFP virus, axons did not make a tight focus (Fig. 3B), converging on two aberrant spots where abnormal arborization and synaptic varicosities were observed. Some axonal trajectories were found to be deflected from their normal path (*11*). From these results, we conclude that misexpression of the *Tbx5* gene in the ventral side of the retina changes the pattern of axonal projection in the tectum.

To investigate this effect further, we also studied axonal projection by retrograde DiI labeling (*2*). DiI dissolved in *N*,*N*-dimethylformamide was injected once with a sharp glass pipette into the ventral tectum to label a small subset of retinal axons (Fig. 3L). After injection, the embryos were incubated for 2 days to allow DiI to be carried back to the retinal ganglion cells, and then dissected retinas were analyzed (Fig. 3K). In control embryos (n = 10), 226 (98%) labeled retinal cells were found in the dorsal retina and 4 (2%) labeled cells were found in the ventral retina (Fig. 3, E and H). After injection of Tbx5-EGFP virus, there was a marked increase of DiI-labeled cells in the ventral retina (31%) (4 out of 10 embryos) (Fig. 3, F and I); 223 DiI signals were found in the dorsal retina, whereas 101 were found in the ventral. Expression of Tbx5 was confirmed by GFP fluorescence (Fig. 3J).

Even with ectopically expressed Tbx5, some ventral retinal axons projected normally to the dorsal side of the tectum. This may have been because ectopic expression of the *Tbx5* gene occurred too late or not all of the retinal cells were infected. In fact, the amount of Tbx5 retrovirus injected was limited, because excessive expression of Tbx5 caused deformities of the brain and premature death of embryos (*16*). Consequently, injected retina did not show ubiquitous expression of Tbx5-EGFP.

Our experiments demonstrate that misexpression of the *Tbx5* gene can induce dorsalization of the eye, suggesting that Tbx5 is involved in the early determination of the D-V axis of developing eye, possibly in conjunction with the dorsally expressed genes *EphrinB1* and *EphrinB2* and the ventrally expressed genes *EphB2*, *EphB3*, *Pax2* (*9*), and *Vax* (*10*). Our data suggest that both Pax2 and Vax act downstream of Tbx5, as both genes were repressed by misexpression of *Tbx5*. However, no effect was observed on the expression of *Pax2* and *Vax* in the optic stalk (*17*). *Pax2* may therefore not be a direct target but may be controlled by other factors in the developing eyes. One candidate could be retinoic acid (RA) because implantation of RA-soaked beads induces ventralization and dorsal *pax[b]* expression in zebrafish eyes (*18*). Other factors may also be involved in the determination of the D-V axis of the eye because dorsalization and repression of *Pax2* and *Vax* were not complete. Such factors could be bone morphogenetic proteins (BMPs), which are also involved in the D-V axis determination (*19*). Moreover, *Drosophila omb* acts in conjunction with *decapen-*

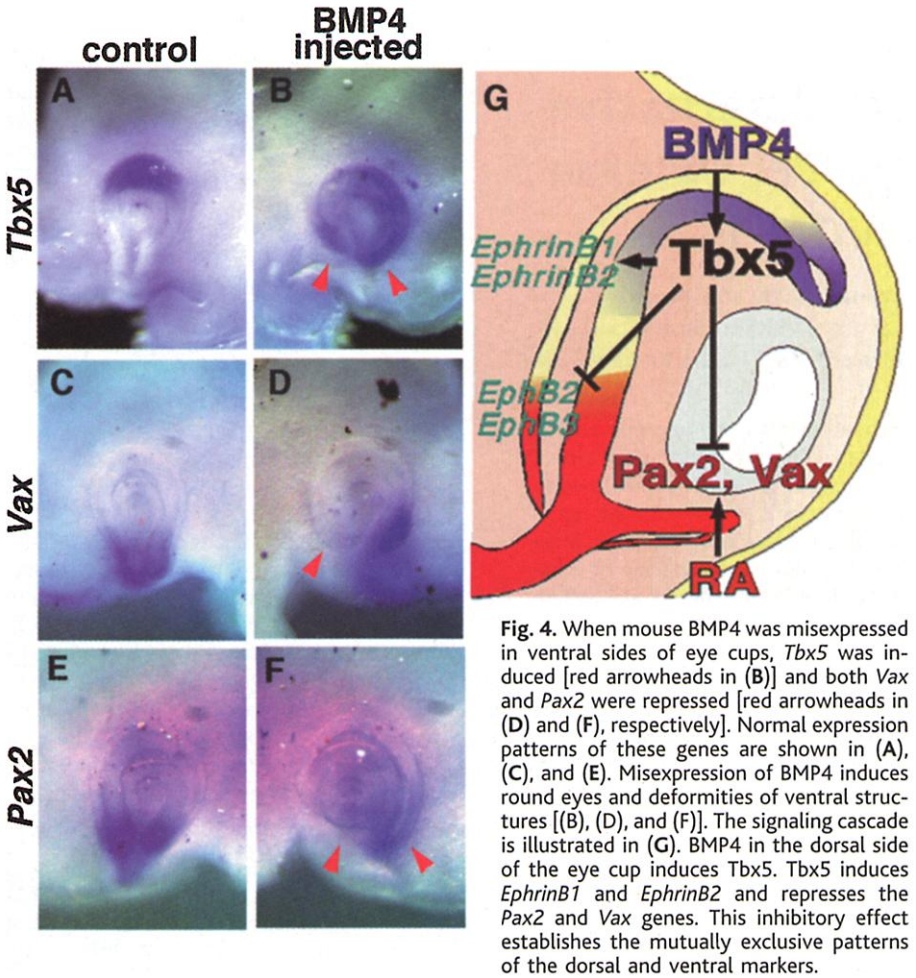

Fig. 4. When mouse BMP4 was misexpressed in ventral sides of eye cups, *Tbx5* was induced [red arrowheads in (**B**)] and both *Vax* and *Pax2* were repressed [red arrowheads in (**D**) and (**F**), respectively]. Normal expression patterns of these genes are shown in (**A**), (**C**), and (**E**). Misexpression of BMP4 induces round eyes and deformities of ventral structures [(B), (D), and (F)]. The signaling cascade is illustrated in (**G**). BMP4 in the dorsal side of the eye cup induces Tbx5. Tbx5 induces *EphrinB1* and *EphrinB2* and represses the *Pax2* and *Vax* genes. This inhibitory effect establishes the mutually exclusive patterns of the dorsal and ventral markers.

taplegic (*dpp*), which belongs to the same family of BMP molecules.

To investigate the functional roles of BMPs, we electroporated CAGGS–mouse *BMP4* in the ventral side of eye cups. When mouse *BMP4* was misexpressed in the ventral half, round eyes were formed with expansion of *Tbx5* expression in the ventral half (Fig. 4, A and B). In such eyes, expression of *Vax* and *Pax2* was repressed (Fig. 4, D and F). As shown in Fig. 4, B, D, and F, misexpression of BMP4 induced more profound effects on eye morphology. These observations indicate that BMP4 acts upstream of Tbx5 (Fig. 4G).

Our results have shown that the *Pax2*, *Vax*, *EphrinB1*, and *EphrinB2* genes act downstream of the *Tbx5* gene. This genetic hierarchy seems in good accordance with the serious expression of *BMP4*, *Tbx5*, *Pax2*, *EphrinB1*, and *EphrinB2* (stage 10+, 11, 11, 13 to 15, and 13 to 15, respectively) (*2*, *3*, *5*, *10*). Misexpression of Tbx5 in the ventral side of the eye induces marked changes of the retinotectum projection without obvious morphological alteration. This rules out the possibility that the effect of *Tbx5* misexpression on the projection is secondary to the dorsalization, because the exit of retinal axons into the optic nerve and the optic nerves themselves were formed normally in virus-infected eyes. Hence, we conclude that the signaling cascade mediated by Tbx5 plays a key role in both eye morphogenesis and the visual projection.

References and Notes

1. A. Szel, P. Rohlich, A. R. Caffe, T. van Veen, *Microsc. Res. Tech.* **35**, 445 (1996); G. A. Hyatt, E. A. Schmitt, J. M. Fadool, J. E. Dowling, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13298 (1996); E. A. Schmitt and J. E. Dowling, *J. Comp. Neurol.* **371**, 222 (1996); A. Szel *et al.*, *J. Comp. Neurol.* **325**, 327 (1992).
2. S. Thanos and F. Bonhoeffer, *J. Comp. Neurol.* **261**, 155 (1987); W. J. Crossland and C. J. Uchwat, *J. Comp. Neurol.* **185**, 87 (1979); J. Mey and S. Thanos, *J. Hirnforsch.* **33**, 673 (1992); M. Jacobson, *Science* **155**, 1106 (1967); J. E. Braisted *et al.*, *Dev. Biol.* **191**, 14 (1997); J. E. Braisted *et al.*, *Dev. Biol.* **191**, 14 (1997); J. A. Holash *et al.*, *Dev. Biol.* **182**, 256 (1997); J. A. Holash and E. B. Pasquale, *Dev. Biol.* **172**, 683 (1995); E. B. Pasquale, *Cell. Regul.* **2**, 523 (1991); J. Yuasa, S. Hirano, M. Yamagata, M. Noda, *Nature* **382**, 632 (1996).
3. J. J. Gibson-Brown *et al.*, *Mech. Dev.* **56**, 93 (1996); S. I. Agulnik *et al.*, *Genetics* **144**, 249 (1996); H. Ohuchi *et al.*, *Development* **125**, 51 (1998).
4. G. O. Pflugfelder *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1199 (1992).
5. J. J. Gibson-Brown, S. Agulnik, L. M. Silver, V. E. Papaioannou, *Mech. Dev.* **74**, 165 (1998); D. L. Chapman *et al.*, *Dev. Dyn.* **206**, 379 (1996).
6. H. Ogino and K. Yasuda, *Science* **280**, 115 (1998); T. Momose *et al.*, *Dev. Growth Differ.* **41**, 335 (1999); J. K. Takeuchi *et al.*, *Nature* **398**, 810 (1999); J. Funahashi *et al.*, *Dev. Growth Differ.* **41**, 59 (1999).
7. Egg shells of stage 8 to 10 embryos were opened, and then membranes were removed to expose the embryos. A cathode and an anode were placed on the left and right sides of the rostral end of the embryos, respectively. Plasmid solutions were injected into the optic vesicles by glass pipettes. Then the electric pulses were applied (voltage: 7 to 9 V; 20 to 50 ms) by the electroporator (BTX, San Diego, CA). Egg shells were sealed and incubated in a humidified incubator.
8. The coding region of chicken *Tbx5* gene was amplified by polymerase chain reaction (PCR) with primers that were designed to fit pSLAX 12 Nco plasmid (*15*) and pEGFP-N2 vector (Clontech). PCR products were subcloned in pSLAX Nco (pSLAX-Tbx5) and pEGFP-N2 vectors (pTbx5-EGFP). pTbx5-EGFP encodes a fusion protein of Tbx5 at the NH_2-terminal and EGFP at the COOH-terminal. A DNA fragment corresponding to Tbx5-EGFP was gel-purified and then subcloned in pSLAX Nco plasmid (pSLAX-Tbx5EGFP). pSLAX-Tbx5 and pSLAX-Tbx5EGFP were restricted with Cla I, and then DNA fragments of *Tbx5* and *Tbx5EGFP* genes were inserted into the Cla I site of modified pCAGGS expression vector (*20*) and RCASBP(A) retrovirus vector as described (*15*).
9. H. O. Nornes *et al.*, *Development* **109**, 797 (1990); P. Sanyanusin *et al.*, *Nature Genet.* **9**, 358 (1995).
10. K. Ohsaki, T. Morimitsu, Y. Ishida, R. Kominami, N. Takahashi, *Genes Cells* **4**, 267 (1999); A. M. Barbieri *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 10729 (1999).
11. See supplementary data at www.sciencemag.org/feature/data/1044053.shl.
12. CsCl-purified retrovirus plasmids (RCASBP-Tbx5/EGFP) were transfected to c/o chicken embryonic fibroblast cells by the calcium phosphate method. Transfected cells were propagated to allow virus production. Then virus stocks were prepared as described (*15*). Virus solution was injected into eye cups at stage 8 to 10. Infection and the expression of Tbx5-EGFP gene were monitored by GFP fluorescence. Both Tbx5 and Tbx5-EGFP genes gave the same results in all experiments.
13. For anterograde DiI labeling (*14*), embryos were incubated for 14 to 16 days after virus injection, and then a tiny DiI crystal was placed in a small spot of the ventral retina. Embryos were incubated for an additional 2 days to allow DiI to label the retinal fibers from the eye to the tectum. Embryos were killed at E18 to E20. Tectums were cut into ventral and dorsal halves and then observed by fluorescence microscopy. Retrograde DiI labeling (*2*) was carried out as described in the text at the same time course as anterograde labeling.
14. H. Nakamura and D. D. O'Leary, *J. Neurosci.* **9**, 3776 (1989).
15. B. A. Motgan and D. M. Fekete, in *Methods in Avian Embryology*, vol. 51 of *Methods in Cell Biology*, M. Bronner-Fraser, Ed. (Academic Press, San Diego, CA, 1996), pp. 185–218.
16. K. Koshiba-Takeuchi, J. K. Takeuchi, T. Ogura, unpublished observation.
17. K. Koshiba-Takeuchi *et al.*, data not shown.
18. A. T. Dudley and E. J. Robertson, *Dev. Dyn.* **208**, 349 (1997); G. A. Hyatt *et al.*, *Development* **122**, 195 (1996).
19. B. L. M. Hogan, *Genes Dev.* **10**, 1580 (1996); A. T. Dudley, K. M. Lyons, E. J. Robertson, *Genes Dev.* **11**, 3254 (1997); Y. Furuta and B. L. M. Hogan, *Genes Dev.* **12**, 3764 (1998).
20. H. Niwa, K. Yamamura, J. Miyazaki, *Gene* **108**, 193 (1991).

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