

- condensed, evenly staining chromosomes were scored as mitotic. At least 300 cells were counted for each determination.
33. Genomic DNA was purified from cell lysates with the QiaAMP spin blood kit (Qiagen) and used as a substrate for polymerase chain reaction and Southern blot assessment of targeting vector integration.
 34. Equal numbers of cells were collected, lysed in Laemmli sample buffer, and subjected to electrophoresis and protein immunoblotting. Filters were probed with antibodies to p53 (pAb 1801) and p21 [EA10 [W. S. El-Deiry *et al.*, *Cancer Res.* 55, 2910 (1995)]]. Signals were visualized with enhanced chemiluminescence (Pierce).
 35. Modified from K. Kaufmann *et al.*, *Cell Growth*.

Diff. 8, 1105 (1997). In brief, extracts for in vitro kinase assays were prepared by lysis of washed, centrifuged cells in 50 mM tris-HCl (pH 7.5), 0.5% Nonidet P-40, 10% (v/v) glycerol, 100 mM sodium chloride, 10 mM sodium orthophosphate, 5 mM β -glycerophosphate, 50 mM sodium fluoride, 0.3 mM sodium orthovanadate, 1 mM dithiothreitol, and 1 \times complete protease inhibitor cocktail (Boehringer Mannheim) for 30 min at 4°C. Kinase complexes were immunoprecipitated by adding monoclonal antibody to cyclin B1 (150 ng, Santa Cruz) and protein A-Sepharose (Life Technologies). Immune complexes were washed with lysis buffer and incubated in 25 μ l of a solution containing 20 mM tris-HCl (pH 7.5), 7.5 mM magnesium chloride,

- 1 mM dithiothreitol, 50 μ M adenosine triphosphate (ATP), 20 μ Ci of [γ - 32 P]ATP (6000 Ci/mmol), and 1 μ g of histone H1 protein (Boehringer-Mannheim) for 30 min at 30°C. After addition of 25 μ l of 2 \times sample buffer and SDS-polyacrylamide gel electrophoresis, 32 P-labeled histone H1 was visualized by autoradiography.
36. Cells were fixed in methanol at -80°C and stained with antibody to γ -tubulin (Sigma) and a fluorescently labeled secondary antibody to mouse immunoglobulin G (Molecular Probes).
37. Supported by the Clayton Fund and NIH grants CA 43460, CA 57345, CA 62924, and GM 41690. We thank G. Wahl for the H2B-GFP fusion vector.

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A Rapidly Evolving Homeobox at the Site of a Hybrid Sterility Gene

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The homeodomain is a DNA binding motif that is usually conserved among diverse taxa. Rapidly evolving homeodomains are thus of interest because their divergence may be associated with speciation. The exact site of the *Ods* locus of hybrid male sterility in *Drosophila* contains such a homeobox gene. In the past half million years, this homeodomain has experienced more amino acid substitutions than it did in the preceding 700 million years; during this period, it has also evolved faster than other parts of the protein or even the introns. Such rapid sequence divergence is driven by positive selection and may contribute to reproductive isolation.

The homeodomain is a stretch of 60 to 62 amino acids that was first discovered to be conserved between many homeotic genes in *Drosophila* (1). Proteins containing this DNA binding motif are usually transcription factors and have been found in most metazoans (2). Evolutionary conservation of homeoboxes has been well documented; for example, the homeodomains in the *Antp* gene of *Drosophila* and grasshoppers have identical sequences (3). Such a high degree of conservation in the protein sequences is often taken as evidence for conservation of the underlying functions. It is thus of great interest to find exceptions in such a highly conservative gene family. Are there homeobox genes that have evolved rapidly and, if there are, what are their functions? What are the selective forces that make them deviate from the norm for this class of genes? It is not inconceivable that their sequence divergence and evolution of the underlying functions may even play a role in differentiation among closely related species. In this report, we describe the cloning of a new homeobox gene that has experienced accelerated evolution in the *Drosophila melanogaster* clade. The acceleration is 100 to 1000

times greater than the rate experienced by its homologs in other taxa. The new homeobox gene was discovered in the search for a "speciation gene" that causes hybrid male sterility.

In a series of studies, several genetic elements responsible for reproductive isolation between *Drosophila simulans* and *Drosophila mauritiana* have been identified (4). One of them, mapped to the cytological interval 16D on the X chromosome, is named *Ods* (5, 6). The introgression of an appropriate *Ods*-containing region of *D. mauritiana* into *D. simulans* renders males completely sterile. The allelic state of *Ods* is nearly fixed in both species (7). In other words, *Ods*-induced hybrid male sterility is observable between any pairwise combination of *D. mauritiana* and *D. simulans* lines.

To delineate the *Ods* locus precisely, we generated 190 new recombinants with progressively shortened introgressions (Fig. 1). With eight molecular markers (8), 63 of these introgressions are male fertile and the remaining 127 are male sterile. In agreement with (6), the distinction between fertile lines (>90% fertility penetrance for each line) and sterile lines (0%) is clear-cut. The two longest fertile introgressions and the two shortest sterile introgressions define the location of the *Ods* locus. Because the breakpoints of the four introgressions all fall within a genomic clone of 8.4 kb (U8 in Fig. 1), it is plausible that the *Ods* gene, or at least part

of it, resides within this clone.

We first obtained the complete DNA sequence of the U8 clone from *D. melanogaster* and used DNA software programs to identify three putative exons. On the basis of the putative exon sequences, we designed polymerase chain reaction (PCR) primers to analyze transcripts in a series of experiments. By the reverse transcriptase-PCR (RT-PCR) procedure, we could detect transcripts of the predicted sizes spanning exons 2 and 3 in both larval and adult stages (8). Then, a near full-length cDNA was obtained from a testis cDNA library by PCR amplification with primers in the exons and in the cloning vector. Finally, the 3' end is determined by the RACE (rapid amplification of cDNA ends) protocol (8). Translation of this cDNA sequence including exon 1, which is located distal to U8, is shown in Fig. 2. The putative protein is 349 amino acids long. Because of the presence of a homeobox in exons 2 and 3, we have named this new transcript *OdsH* (for *Ods*-site homeobox gene). The name implies the correspondence in position between the genetic and molecular data without stating their functional equivalence.

The best and highly significant matches with *OdsH* in the database are the *unc-4* gene of *Caenorhabditis elegans*, *uncx4.1* of mouse (and its rat homolog), and an unpublished sequence from planaria (9). These sequences comprise a homologous cluster belonging in the paired-type subfamily of homeobox genes. Some of these homologous genes from very divergent taxa—notably *Drosophila*, rodents, and planaria—also have significant matches beyond the homeodomain. For example, 13 of the 14 amino acids adjoining the COOH-terminus of the homeodomain are identical in mouse and *Drosophila*. A high level of conservation extends for 33 amino acids from the COOH-terminus. In this report, we focus on exons 2 and 3 because their products contain the conservative homeodomain, which would allow us to contrast long-term evolutionary stability (such as between mammals and *Drosophila*) with recent rapid changes (between sibling species). Homology with non-*Drosophila* species outside these two exons is too low to be informative.

In comparing homologous genes from dif-

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ferent species, it is important to distinguish between orthologous and paralogous sequences. The former share a common ances-

try due to speciation and the latter by gene duplication. Paralogous genes from different species must have diverged for a longer time

than the orthologous copies. The mouse and *C. elegans* homeodomains in Fig. 3A have diverged at only 7 sites of the 60 residues. Between the mammalian homolog and *OdsH* of *D. melanogaster*, there are 17 differences.

Although the homologs of *OdsH* appear conserved among distantly related taxa like mammals and *Drosophila*, *OdsH* has evolved rapidly among sibling species of the *D. melanogaster* clade. Amino acid sequences corresponding to exons 2 and 3 of *OdsH* in *Drosophila* species are presented in Fig. 3A. *D. simulans*, *Drosophila sechellia*, and *D. mauritiana* are close enough to produce fertile F₁ females (10) and are estimated to have diverged for about half a million years (11). Each of the trio can also be crossed to *D. melanogaster* (10), which probably diverged from the trio about 1 million years ago (12, 13). We sequenced multiple alleles from each species to ensure that no anomaly is associated with sampling or laboratory procedures. Within-species variations (or lack of) will be useful for future population genetic analysis.

The most visually striking feature of Fig. 3 is the surge of amino acid changes between these sibling species within the homeodomain itself. Between *D. simulans* and *D. mauritiana*, the species pairs analyzed for the *Ods*-induced hybrid sterility, there are 15 amino acid differences in the homeodomain of *OdsH*. This difference is almost as large as that between *OdsH* of *Drosophila* and *uncx4.1* of mouse and it is larger than that between *C. elegans* and mouse. For a comparison with another closely related species pair, the homologs between mouse and rat have only 1 amino acid difference after more than 20 million years of divergence (14). It should be noted that the acceleration in *OdsH* is much greater than in other well-known fast-evolving homeoboxes; for example, the *ftz* homeobox differs by 10 or 11 amino acids in different orders of insects (2), less divergent than the *OdsH* of *Drosophila* sibling species.

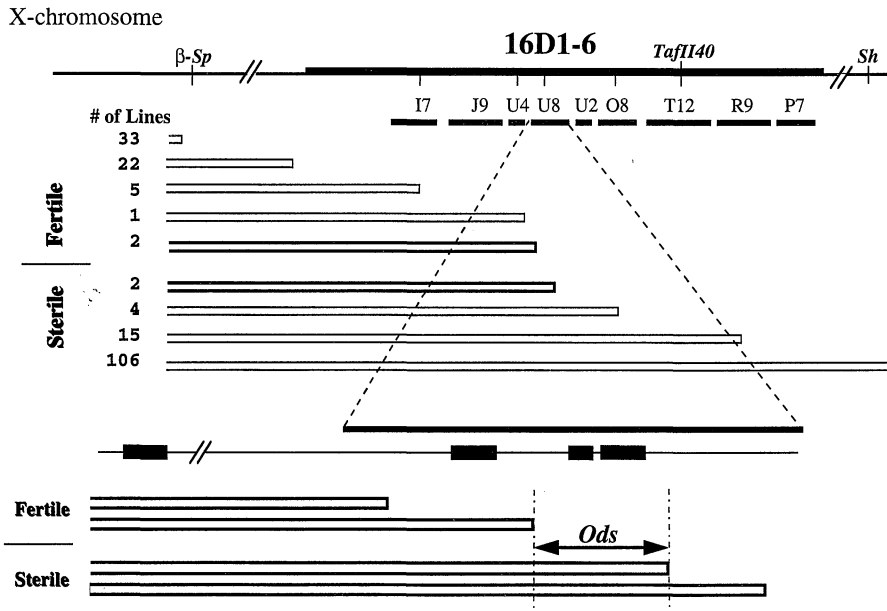


Fig. 1. Molecular mapping of the *Ods* gene. Thick line represents cytological interval 16D covered by two P1 phage clones; line segments below indicate subclones, with the number denoting their size in kilobases. Open boxes represent introgressions from *D. mauritiana*. Introgressions are grouped according to size and the number of lines in each size class is given. The difference between the fertile and sterile introgressions is mapped to the U8 clone. Three of the four exons of the *OdsH* transcript are contained within U8.

Fig. 2. Amino acid sequence of *OdsH* translated from the testis cDNA clone of *D. melanogaster*. The homeodomain is underlined and the intron positions are indicated with arrowheads. The first methionine was putatively identified by analyzing DNA sequences from both *D. melanogaster* and *D. mauritiana*. GenBank accession number for the cDNA sequence is AF095575.

MQVSGWSSVLNLSMDAPNPEISPNSTSSSVYIMIRQMALIQARVAAAA
VAMQQQQQQQQQQQDLNRELGMDFHSEQRKLDVSPTNHNHAGSSRGI
KQDPLSDEGADSNLQNDCTESSKKRRGRNTFNFSWQLRELRFVFGSHYP
DIFMREALATKLDLMEGRIAVWFONRRAKWRKOEHTKKGPGRPAHNAHPQ
SCSGDPIPLSELRLARELAQRSKRMKKAIDRQAKKLQDKGLEVDYARLEAE
YLAVHQENGVDENNWLDDDDGYDDLHIDVVGVEPEYVTGDSLDSHFCSSRT
YQTKSTSSELDSDNDMGLQGRVETPPPPQPPMQNKTLYNSFFSIESLLGS.

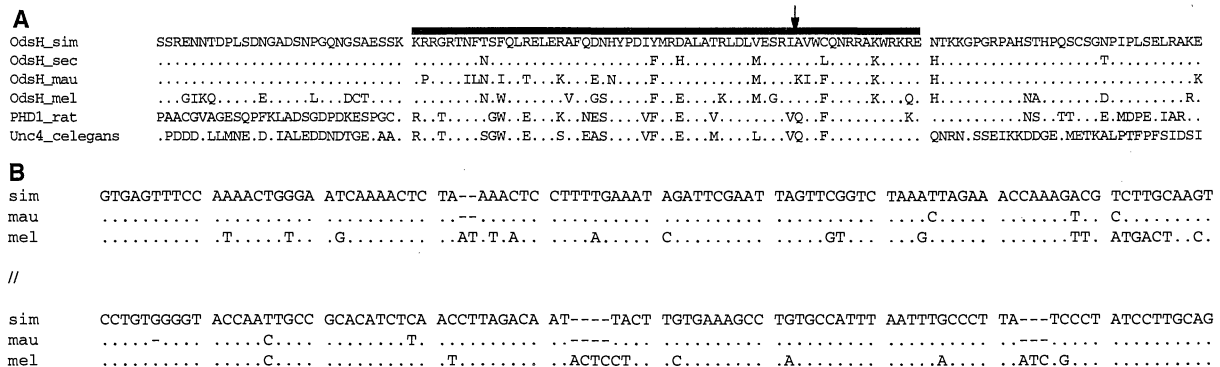


Fig. 3. (A) Amino acid sequence of exons 2 and 3 from four sibling species of *Drosophila*: *D. simulans* (sim), *D. sechellia* (sec), *D. mauritiana* (mau), and *D. melanogaster* (mel). Multiple alleles (three to six) from each species are sequenced. With the exception of *D. simulans*, which has three polymorphic sites, all species are invariant in their amino acid sequence. Arrow indicates

intron position and thick bar covers the homeodomain. *Ods* homologs from rat and *C. elegans* are also indicated. Alignment outside the homeodomain is not always possible. (B) Sequences of the intron between exons 2 and 3 from the three species of *Drosophila*. Only 100 base pairs contiguous with either exon 2 or exon 3 are presented.

Finally, the homeodomain appears to have evolved more rapidly than the remaining part of the protein or the adjoining intron sequences between *D. simulans* and *D. mauritiana* (Fig. 3).

Given the large differences in the *OdsH* sequences of the sibling species, it is imperative to show that these genes are orthologous. Introns and the surrounding noncoding sequences were obtained from the corresponding genomic regions. Two short stretches of sequence from both ends of the intron between exons 2 and 3 are shown in Fig. 3B (the whole 1.3 kb has been analyzed as indicated in Table 1). The orthologous relationship is unambiguous. The difference between *D. simulans* and *D. mauritiana* in this entire intron of 1.3 kb is 1.44%, which agrees very well with the average value for other noncoding sequences at 1% to 2% between this species pair (11, 13).

We now ask whether *OdsH* has become a nonessential gene in *Drosophila* with relaxed selection or whether positive selection for favorable amino acids has been driving the rapid evolution. From the results of Table 1, the rate of nucleotide substitution in the homeodomain between *D. simulans* and *D. mauritiana* is 8 times higher than that in the intron, which

should be close to the neutral rate. The difference is highly significant ($P < 0.01$ by Fisher's exact test) and is most compatible with the positive-selection interpretation (15). On the other hand, the rate in the nonhomeodomain is slightly less than the intron rate. In the comparisons between more distantly related pairs involving *D. melanogaster*, the substitution rates in the homeodomain are only twice as high as those in the intron but the nonhomeodomain appears to have evolved rapidly as well. Therefore, a conservative conclusion is that negative selection on *OdsH* has generally been relaxed in the *D. melanogaster* clade but positive selection can be inferred in some cases, such as in the homeodomain of *D. mauritiana* and *D. simulans*.

In a more detailed analysis, we compare the number of nucleotide substitutions that result in amino acid replacements (R) and those that are silent (S) along each branch (8). The R/S numbers in the homeodomain are 10:1 in the branch leading to *D. mauritiana* but 10:9 in the branch between node A of Fig. 4 and *D. melanogaster*. The difference is significant ($P < 0.05$ by Fisher's exact test), which suggests an excess in replacements in *D. mauritiana* relative to that in

D. melanogaster. We may also compare the replacement numbers in the homeodomain and nonhomeodomain, both about 60 residues, between species. The replacement numbers in the two domains are 10:1 in the *D. mauritiana* lineage and 10:15 in the *D. melanogaster* lineage ($P < 0.01$), which suggests that the selective pressure in the homeodomain, relative to that in the nonhomeodomain, is very different in these two species. This test also reveals a marginally significant difference between *D. simulans* and *D. melanogaster* (6:1 and 10:15, respectively, with $P = 0.041$ in the one-tailed test). Apparently, the homeodomain has been evolving rapidly in all three *Drosophila* species but the acceleration is most dramatic in *D. mauritiana*.

What may be the nature of the positive selection that drives the evolution of *OdsH*? We note that the putative homologs of *OdsH* in mammals and nematodes function in neural tissues (9) but *OdsH* is also expressed in the *Drosophila* testis. It is plausible that the accelerated evolution of *OdsH* is concomitant with its acquisition of a male germ line function. [There is preliminary evidence for ancient duplication of *OdsH* in *Drosophila* and this paralogous gene expresses in neural tissue (16); therefore, *OdsH* could have been selected for new functions.] Male reproductive function is sometimes accompanied by the opportunities for sexual selection driving rapid sequence evolution (17). If the hypothesis is correct, one would expect to observe accelerated evolution in species whose *OdsH* homolog has acquired male germ line expression. Whatever the cause of the rapid amino acid substitution, a by-product of these changes could be hybrid male sterility. Because males bearing either fertile or sterile introgressions indicated in Fig. 1 differ only in the species origin of the region of exons 3 and 4 of *OdsH*, which, moreover, is expressed in both fertile and sterile males, the cause(s) of hybrid sterility must be primarily in the amino acid sequences of these exons.

The studies of speciation among closely related species have barely entered the molecular era—notably in marine invertebrate systems (18). At the other end of the spectrum, homeobox genes have been extensively studied but mostly among very distantly related taxa (19). Molecular characterization of genes like *OdsH* may allow us to combine speciation studies and molecular evolution analysis into a coherent discipline.

Fig. 4. Summary of sequence evolution in *OdsH* and its homologs. (A) Numbers of inferred amino acid changes in the homeodomain are indicated next to the branches. Assignment is according to the method of Fitch (20). Because of multiple nucleotide substitutions within each codon, the numbers of nonsynonymous nucleotide substitutions underlying these amino acid changes are 8.4 (to *C. elegans*), 8.1 (to the mouse/rat ancestor), and 20.6 (to *Drosophila*). (B) Numbers of amino acid changes in the homeodomain are indicated next to the branches as in (A). All three branches are between node A and the extant species. Numbers of nucleotide substitutions underlying these amino acid replacements in the homeodomain are indicated in the first row below (R). The numbers of silent nucleotide changes are also indicated (S). For comparisons, changes in the nonhomeodomain are also indicated.

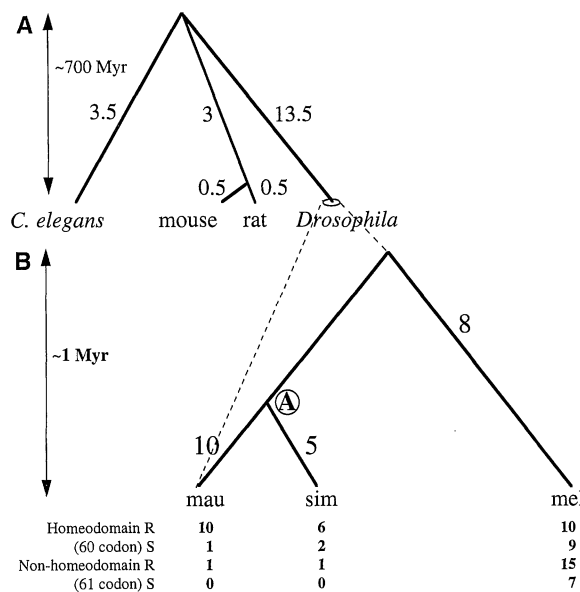


Table 1. Divergence in the homeodomain-containing exons of *OdsH* between sibling species of *Drosophila*. Numbers are estimated substitutions per base pair (bp) (21). Most nucleotide changes in the exons are nonsynonymous (see Fig. 4). Sim, *D. simulans*; mau, *D. mauritiana*; mel, *D. melanogaster*.

	Exons 2 and 3		Intron between exons 2 and 3 (1300 bp)
	Homeodomain (180 bp)	Nonhomeodomain (183 bp)	
sim-mau	0.110 ± 0.043	0.011 ± 0.013	0.014 ± 0.005
mel-sim	0.159 ± 0.052	0.129 ± 0.046	0.078 ± 0.013
mel-mau	0.178 ± 0.055	0.129 ± 0.046	0.080 ± 0.014

References and Notes

- W. McGinnis, R. L. Garber, J. Wirz, A. Kuroiwa, W. J. Gehring, *Cell* **68**, 283 (1984); M. P. Scott and A. J. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4115 (1984).
- T. R. Burglin, in *Guidebook to the Homeobox Genes*, D. Duboule, Ed. (Oxford University Press, Oxford, 1994), pp. 27–71; R. Dawes, I. Dawson, F. Falciani, G. Tear, M. Akam, *Development* **120**, 1561 (1994).
- D. C. Hayward, N. H. Patel, E. J. Rehm, C. S. Goodman, E. E. Ball, *Dev. Biol.* **172**, 452 (1995).
- E. L. Cabot, A. W. Davis, N. A. Johnson, C.-I. Wu,

- Genetics* **137**, 175 (1994); M. Palopoli and C.-I. Wu, *ibid.* **138**, 329 (1994); J. R. True, B. S. Weir, C. C. Laurie, *ibid.* **142**, 819 (1996); H. Hollocher and C.-I. Wu, *ibid.* **143**, 1243 (1996).
5. D. E. Perez, C.-I. Wu, N. A. Johnson, M.-L. Wu, *ibid.* **134**, 261 (1993).
6. D. P. Perez and C.-I. Wu, *ibid.* **140**, 201 (1995).
7. D. E. Perez, thesis, University of Chicago (1995).
8. The RT-PCR procedure has been done on all three species of *Drosophila* indicated in Fig. 4. For technical details, refer to the supplementary material at www.sciencemag.org/feature/data/983600.
9. D. M. Miller et al., *Nature* **355**, 841 (1992) (GenBank accession number X64904); A. Mansouri et al., *Dev. Dynamics* **210**, 53 (1997) (GenBank accession number Z96107); T. Saito, L. Lo, D. J. Anderson, K. Miko-shiba, *Dev. Biol.* **180**, 143 (1996) (GenBank accession number D87748); A. M. Munoz-Marmol et al., GenBank accession number Y10299.
10. F. Leméunier, J. R. David, L. Tsacas, M. Ashburner, in *The Genetics and Biology of Drosophila*, M. Ashburner, H. L. Carson, J. N. Thompson, Eds. (Academic Press, New York, 1986), vol. 3e, pp. 148–256.
11. J. Hey and R. M. Kliman, *Mol. Biol. Evol.* **10**, 804 (1993).
12. F. J. Ayala and D. L. Hartl, *ibid.*, p. 1030.
13. A. Caccione, E. N. Moriyama, J. M. Gleason, L. Nigro, J. R. Powell, *ibid.* **13**, 1224 (1996).
14. S. Kumar and S. B. Hedges, *Nature* **392**, 917 (1998).
15. J. Zhang, H. Rosenberg, M. Nei, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3708 (1998).
16. K. Tabuchi, S. Yoshikawa, M. Okabe, K. Sawamoto, H. Okano, paper presented at the 39th Annual *Drosophila* Research Conference, Washington, DC, 25 to 29 March 1998, p. 545C (abstr.).
17. W. G. Eberhard, *Sexual Selection and Animal Genitalia* (Harvard University Press, Cambridge, MA, 1985); S. Maiti et al., *Genomics* **34**, 304 (1996). C.-I. Wu, N. A. Johnson, M. F. Palopoli, *Trends Ecol. Evol.* **11**, 281 (1996); S. C. Tsaur and C.-I. Wu, *Mol. Biol. Evol.* **14**, 544 (1997); S. C. Tsaur, C.-T. Ting, C.-I. Wu, *ibid.* **15**, 1040 (1998); A. Civetta and R. Singh, *ibid.*, p. 901.
18. Y.-H. Lee, T. Ota, V. D. Vacquier, *Mol. Biol. Evol.* **12**, 231 (1995); E. C. Metz and S. R. Palumbi, *ibid.* **13**, 397 (1996). W. J. Swanson and V. D. Vacquier, *Science* **281**, 710 (1998); C.-I. Wu and M. F. Palopoli, *Annu. Rev. Genet.* **28**, 283 (1994).
19. S. B. Carroll, *Development Suppl.*, 217 (1994). M. Averof and N. Patel, *Nature* **388**, 682 (1997).
20. W. M. Fitch, *Syst. Zool.* **20**, 406 (1971).
21. T. H. Jukes and C. R. Cantor, in *Mammalian Protein Metabolism*, H. N. Munro, Ed. (Academic Press, New York, 1969).
22. Supported by National Science Foundation and National Institutes of Health grants to C.-I.W. We are indebted to M. Itoh for collaborating with us in construction of the *D. simulans* and *D. mauritiana* genomic libraries and to T. Hazelrigg for sending us the testis cDNA library. We also thank C. H. Langley, N. Patel, M. F. Polopalli, I. Boussey, C. M. Bergman, P. Andolfatto, M. Long, and S. M. Rollmann for technical consultations and helpful discussions.

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Local GABA Circuit Control of Experience-Dependent Plasticity in Developing Visual Cortex

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Sensory experience in early life shapes the mammalian brain. An impairment in the activity-dependent refinement of functional connections within developing visual cortex was identified here in a mouse model. Gene-targeted disruption of one isoform of glutamic acid decarboxylase prevented the competitive loss of responsiveness to an eye briefly deprived of vision, without affecting cooperative mechanisms of synapse modification in vitro. Selective, use-dependent enhancement of fast intracortical inhibitory transmission with benzodiazepines restored plasticity in vivo, rescuing the genetic defect. Specific networks of inhibitory interneurons intrinsic to visual cortex may detect perturbations in sensory input to drive experience-dependent plasticity during development.

After even a brief period of monocular occlusion in early life, input to visual cortex from the closed eye is functionally weakened, then anatomically reduced in size (1). How perturbed levels of neuronal activity are detected to produce changes in connectivity within cortex remains unknown. Inhibitory interactions between inputs may play a role (2), as supported by the profound effects on cortical activity of pharmacologically manipulating γ -aminobutyric acid (GABA) receptors during a period of monocular deprivation. GABA_A agonists induce a robust reverse shift in favor of the

deprived eye (3), whereas antagonists provide mixed results, disrupting plasticity in some cases but yielding no effect under other conditions sufficient to produce continuous epileptiform activity (4). Moreover, these gross drug treatments that shut down or hyperexcite the cortex offer little insight into the normal function of intrinsic networks of inhibitory interneurons during visual cortical plasticity.

Mouse models deficient in the enzymes that produce GABA provide an opportunity to address the role of endogenous inhibitory transmission in cortical plasticity. Distinct genes encode two isoforms of the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD). The larger 67-kD protein (GAD67) is localized to cell somata and dendrites, providing a constitutive concentration of GABA throughout the cell by a transporter release mechanism (5). In the absence of GAD67, mice die at birth with GABA concentrations less than 10% of those found in the brains of wild-type littermates (6). The 65-kD isoform (GAD65) is

found primarily in the synaptic terminal (7), where it is anchored to vesicles and serves as a reservoir of inactive GAD that can be recruited when additional GABA synthesis is required (8). The smaller isoform of GAD may therefore be specialized to respond to rapid changes in synaptic demand during intense neuronal activity.

We hypothesized that a loss of the GAD65 isoform would selectively reduce fast, intrinsic inhibitory transmission. The deleterious pathology of completely blocking GABAergic synapses pharmacologically could thus be avoided. Indeed, mice carrying a targeted disruption of the GAD65 gene (GAD65 KO mice) survive and develop typical gross cortical morphology and normal adult GABA concentrations because the expression of GAD67 is unaltered (9). Biochemical analysis of various brain regions (Fig. 1A) revealed that unlike in the adult, GAD65 contributes significantly to total GABA concentrations during early postnatal development (10). Consistent with our results, GAD65 expression is elevated in wild-type (WT) animals younger than 3 weeks of age during periods of active synaptogenesis (11). To determine whether the stimulated release of GABA is in fact compromised by loss of the GAD65 protein, we used microdialysis to directly monitor GABA output from the binocular zone of visual cortex in vivo (12). Although the basal extracellular concentrations were stable and similar between mutant and wild-type mice, maximal GABA release in response to a brief high-potassium depolarization was significantly reduced in GAD65 KO mice (Fig. 1B).

A physiological consequence of GAD65 deficiency in the visual cortex was enhanced activation in response to visual stimulation. Neurons modulate the expression of certain immediate-early genes in response to sensory stimuli (13). GAD65 KO mice at the peak of the critical period for plasticity exhibited a supernormal sensitivity to visual stimulation after an initial period of dark-rearing (Fig. 1C), as as-

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