

29. Streit, A. & Stern, C. D. Mesoderm patterning and somite formation during node regression: differential effects of chordin and noggin. *Mech. Dev.* **85**, 85–96 (1999).  
 30. Stern, C. D. Detection of multiple gene products simultaneously by in situ hybridization and immunohistochemistry in whole mounts of avian embryos. *Curr. Top. Dev. Biol.* **36**, 223–243 (1998).

**Acknowledgements**

We thank A. Rosenthal and W. Ye (Genentech) for the FGFR1-IgG construct; R. Lovell-Badge for *Sox3* and *Sox2* and T. Jessell for S17; C. Dulac for advice on the differential screen; B. Cigich for technical assistance; I. Skromne for Fig. 4b, c; C. Ang for zebrafinch tissue; and T. Jessell, G. Sheng, K. Storey and D. Vasiliauskas for helpful comments on the manuscript. Supported by the National Institute of Mental Health.

Correspondence and requests for materials should be addressed to C.D.S. (e-mail: cds20@columbia.edu).

**Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2**

Miyoko Higuchi\*, Stefan Maas\*†‡, Frank N. Single\*†, Jochen Hartner\*, Andrei Rozov§, Nail Burnashev§, Dirk Feldmeyer§, Rolf Sprengel\* & Peter H. Seeburg\*

\* Departments of Molecular Neurobiology and § Cell Physiology, Max-Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany

† These authors contributed equally to this work

RNA editing by site-selective deamination of adenosine to inosine<sup>1,2</sup> alters codons<sup>3,4</sup> and splicing<sup>5</sup> in nuclear transcripts<sup>6</sup>, and therefore protein function. ADAR2 (refs 7, 8) is a candidate mammalian editing enzyme that is widely expressed in brain and other tissues<sup>7</sup>, but its RNA substrates are unknown. Here we have studied ADAR2-mediated RNA editing by generating mice that are homozygous for a targeted functional null allele. Editing in *ADAR2*<sup>-/-</sup> mice was substantially reduced at most of 25 positions in diverse transcripts<sup>3–6</sup>; the mutant mice became prone to seizures and died young. The impaired phenotype appeared to result entirely from a single underedited position, as it reverted to normal when both alleles for the underedited transcript were substituted with alleles encoding the edited version exonically<sup>9</sup>. The critical position specifies an ion channel determinant<sup>10</sup>, the Q/R site<sup>3,6</sup>, in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptor<sup>10</sup> GluR-B pre-messenger RNA. We conclude that this transcript is the physiologically most important substrate of ADAR2.

Mammalian transcripts that are known to be edited by site-selective adenosine deamination are expressed largely in brain: most encode subunits of ionotropic glutamate receptors (GluRs) that mediate fast excitatory neurotransmission<sup>3,10</sup>. The only position edited to nearly 100% is the Q/R site of GluR-B, for which the mRNA contains an arginine (R) codon (CIG) in place of the genomic glutamine (Q) codon (CAG)<sup>3</sup>. The physiological importance of this codon substitution wrought by RNA editing was revealed by early onset epilepsy and premature death of mice heterozygous for an intron-11-modified *GluR-B*<sup>ΔECS</sup> allele with Q/R site-uneditable transcripts<sup>11,12</sup>.

Three mammalian adenosine deaminases acting on RNA

(ADAR1–ADAR3; refs 7, 8, 13) form a small family of candidate RNA-editing enzymes that operate on nuclear transcripts. Only ADAR2 edits the Q/R site in GluR-B pre-mRNA efficiently *in vitro*<sup>7,14,15</sup>. Because ADAR2 is expressed in tissues other than brain<sup>7</sup>, distinct pre-mRNAs in different tissues may be substrates for this enzyme. To determine whether ADAR2 edits the Q/R site in GluR-B pre-mRNA *in vivo*, and to evaluate the general physiological significance of ADAR2-mediated RNA editing, we generated mice with functional null alleles for this enzyme.

A targeting vector for functional *ADAR2* gene ablation (Fig. 1a, b) was constructed by replacing most of exon 4 (ref. 16) with a *PGK-neo* gene; exon 4 encodes an essential adenosine deaminase motif<sup>3,7</sup>. Chimaeric mice were generated by injection of a targeted embryonic stem (ES) cell clone<sup>17</sup> into C57BL/6-derived blastocysts. *ADAR2*<sup>+/-</sup> intercrosses produced *ADAR2*<sup>-/-</sup> mice at Mendelian frequency, indicating that ADAR2 deficiency does not interfere with embryonic development. We found residual expression from the targeted *ADAR2*<sup>-</sup> allele through exon skipping, potentially leading to a truncated, enzymatically inactive ADAR2 form with intact RNA-binding domains (Fig. 1c). The expression of this truncated form might amount to less than 10% of ADAR2 in wild-type mice, as predicted from the severely reduced mutant transcript levels (mean ± s.d., 8 ± 3% of wild type, *n* = 3; postnatal day 14 (P14)) determined by ribonuclease (RNase) protection (Fig. 1d).

Heterozygous *ADAR2*<sup>+/-</sup> mice were phenotypically normal, but *ADAR2*<sup>-/-</sup> mice died between P0 and P20 and became progressively seizure-prone after P12, akin to *GluR-B*<sup>+ΔECS</sup> mice<sup>11,12</sup>. Thus, we first studied the effect of ADAR2 deficiency on Q/R site editing of GluR-B pre-mRNA, the substrate for a nuclear RNA-dependent

**Table 1 ADAR2 deficiency and site-selective adenosine deamination**

Editing sites	ADAR2 <sup>+/+</sup>	ADAR2 <sup>+/-</sup>	ADAR2 <sup>-/-</sup>
GluR-B pre-mRNA			
Q/R*	98 ± 3	90 ± 3	10 ± 3
Hotspot1*†	50	45	60
Hotspot2*†‡			
+262	30	20	<10
+263	65	55	<10
+264	15	10	<5
GluR-B mRNA			
Q/R*	100 ± 1	100 ± 1	40 ± 4
AMPA-R† R/G§			
GluR-B	75	55	15
GluR-C	90	85	75
GluR-D	45	40	10
GluR5			
Q/R	64 ± 5	55 ± 2	40 ± 1
GluR6			
Q/R	86 ± 4	78 ± 4	29 ± 8
I/V	87 ± 2	79 ± 7	22 ± 5
Y/C	90 ± 4	82 ± 5	2 ± 1
5HT2C-R†			
A	75	70	70
B	80	75	30
C	15	10	<5
D	70	55	<5
ADAR2†‡			
-1	15	10	<10
+23	25	15	<10
+24	45	35	10

The editing sites have been described<sup>2–5,27</sup>. Values, given as mean ± s.d. (*n* = 3), indicate the percentage of the edited version for the different editing sites analysed in the three genotypes. Values were obtained from whole-brain RNA by differential oligonucleotide-mediated hybridization of cloned RT-PCR products to distinguish between an adenosine (unedited) and a guanosine (edited) at the individual editing sites.

\* Values from P14 mice with unmodified *GluR-B* alleles. All other values were from P40 mice with *GluR-B* alleles sequence modified at the Q/R site codon<sup>9</sup>.

† Values derived from the different peak heights for the two nucleotides in identical positions in DNA sequence chromatograms. Values from three mice were averaged and rounded to the nearest 5 or 0 position in each case.

‡ Sites +265 in hotspot2 and -2, +9 and +10 in ADAR2 were edited to <5% in wild type.

§ Flip and flop splice versions. As assessed from sequence chromatograms, the approximate representations of the flip forms were 50% for GluR-B, 75% for GluR-C and 65% for GluR-D; see ref. 27.

‡ Present address: Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, MA 02139, USA.

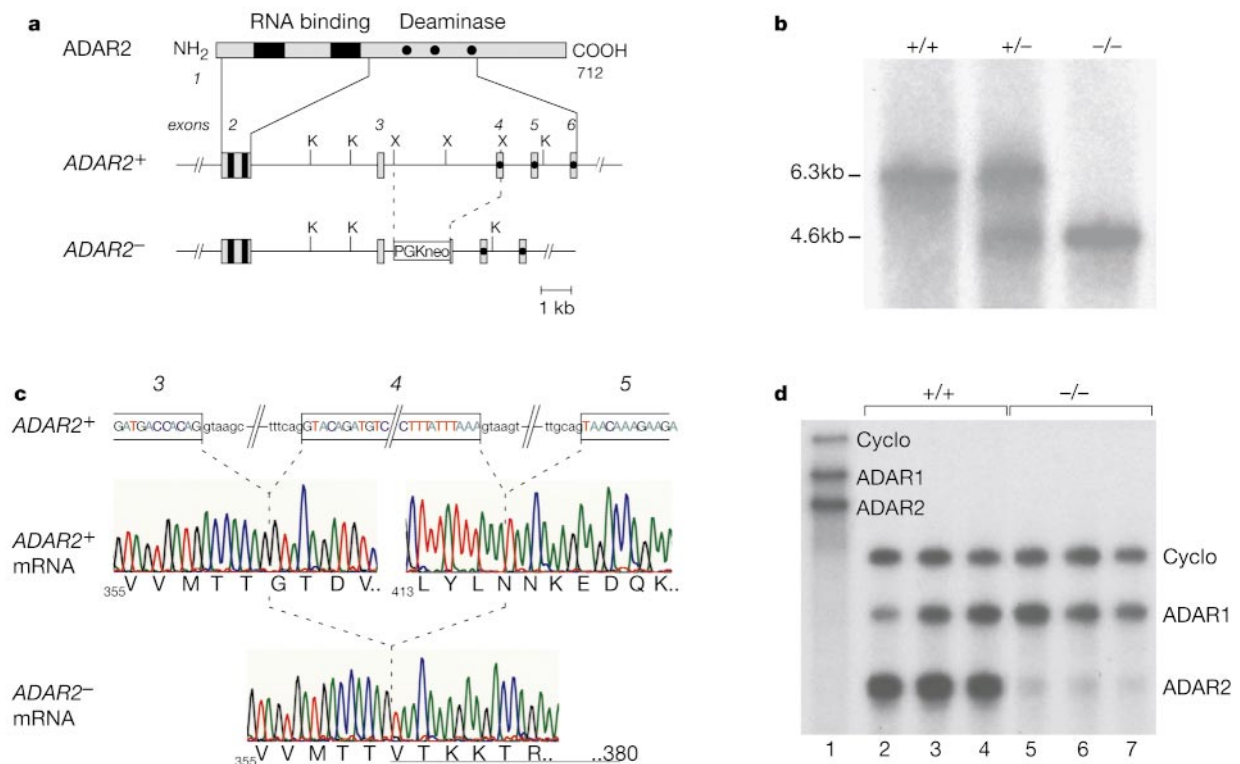
adenosine deaminase activity<sup>6</sup>. As determined from cloned polymerase chain reaction with reverse transcription (RT-PCR) products from brain RNA<sup>6</sup>, Q/R site editing in primary GluR-B transcripts was tenfold lower in *ADAR2*<sup>-/-</sup> than in wild-type mice (10% compared with 98%, Table 1). This identifies ADAR2 as the principal RNA-editing enzyme at the Q/R site. The remaining low level of Q/R site editing in GluR-B pre-mRNA cannot be mediated by the residual, enzymatically inactive, truncated ADAR2 protein, but is mediated by another ADAR, perhaps ADAR1 (refs 14, 18), for which gene expression appeared unchanged in *ADAR2*<sup>-/-</sup> mice (Fig. 1d).

The low extent of Q/R site editing of GluR-B pre-mRNA led to nuclear accumulation of incompletely processed primary GluR-B transcripts and to a fivefold reduction in GluR-B mRNA, as assessed by RNase protection (20 ± 5%; *n* = 3; Fig. 2a) and quantitative RT-PCR (20 ± 4% of wild type; *n* = 3; P14; not shown). The increased level of intron 11-containing GluR-B transcripts and the decrease in GluR-B mRNA were easily visualized by *in situ* hybridization (Fig. 2b). Editing is thus a prerequisite for efficient splicing and processing of the pre-mRNA. The edited GluR-B transcripts are preferentially spliced, as revealed by a shift in Q/R site editing from 10% to 40% when comparing intron-11-containing transcripts with GluR-B mRNA (Table 1). A defect in transcript processing caused by the interaction of the residual truncated ADAR2 protein with RNA can be excluded because GluR-B pre-mRNA accumulation is also

observed in *ADAR2*<sup>+/-</sup> mice expressing the Q/R site-uneditable *GluR-B*<sup>ΔECS</sup> allele<sup>11</sup>.

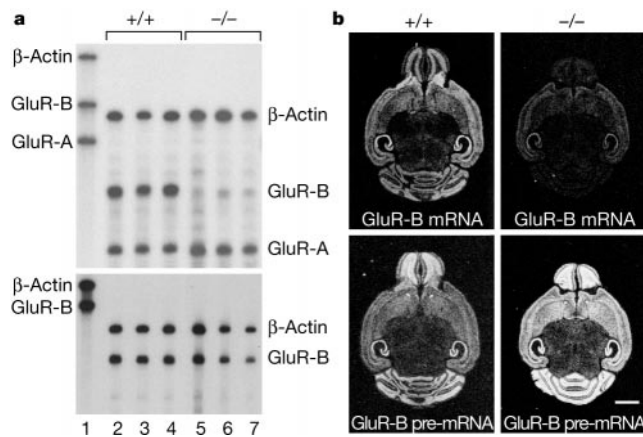
The fivefold drop in mRNA reduced GluR-B subunit levels similarly (21 ± 3% of wild type; *n* = 3; P14), as indicated by western analysis of brain protein from *ADAR2*<sup>-/-</sup> mice (Fig. 3a), and as visualized by immunocytochemistry in the hippocampal subfields (Fig. 3b). We also observed a moderate reduction in GluR-A protein (42 ± 5% of wild type; *n* = 3; Fig. 3a), barely detected by immunocytochemistry (Fig. 3b), with GluR-A mRNA being only slightly reduced (RNase protection: 83 ± 10% of wild type; *n* = 3; P14; Fig. 2a). The lower GluR-A protein levels may be a consequence of the severe reduction in GluR-B, the principal partner of GluR-A in AMPA receptor assembly<sup>9</sup>. Notably, mice lacking all GluR-A expression are phenotypically robust and normal in most respects<sup>19</sup>, precluding the possibility that the moderate GluR-A reduction contributes to the severe phenotype of *ADAR2*<sup>-/-</sup> mice. NMDA receptor<sup>10</sup> NR1 protein levels in *ADAR2*<sup>-/-</sup> mice appeared unchanged (data not shown).

As predicted from previous studies<sup>11,12,20</sup>, reduced expression and reduced Q/R site editing of GluR-B alters AMPA receptor channel properties mainly in principal neurones, which normally express fully edited GluR-B at levels sufficient for incorporation into most heteromeric AMPA receptors. Indeed, in accordance with the molecular analysis, AMPA receptor-mediated currents recorded in acute *ADAR2*<sup>-/-</sup> brain slices from nucleated patches of CA1



**Figure 1** Targeted *ADAR2* allele (Mouse Genome Database symbol, *Adarb1*) and transcript. **a**, Domain structure of the RNA-dependent adenosine deaminase ADAR2 and exon-intron organization of *ADAR2*<sup>+</sup> and *ADAR2*<sup>-</sup> alleles. Protein domains<sup>3,7</sup> are connected to their corresponding exons<sup>16</sup>. In the *ADAR2*<sup>-</sup> allele, most of intron 3 and exon 4 is replaced by *PGK-neo* (dashed lines). Filled boxes, double-stranded RNA-binding domains in protein and exons; filled circles, conserved sequences in deaminase domain of ADARs<sup>3</sup>. X, *XhoI*; K, *KpnI*. **b**, Southern analysis of *ADAR2* alleles in *KpnI*-restricted genomic DNA from mouse liver with a cDNA probe encoding residues 323–452 from exons 3–5. +/+, wild type; +/-, heterozygote; -/-, *ADAR2*-deficient homozygote. **c**, DNA sequence analysis of RT-PCR products from *ADAR2*<sup>+</sup> and *ADAR2*<sup>-</sup> mRNAs. A segment with exons 3–5 of the *ADAR2*<sup>+</sup> allele depicts nucleotides coloured as in chromatograms for wild-type

and mutant cDNA sequences. Amino acids are given as single letters, numbered according to ADAR2. Exon sequence deleted in *ADAR2*<sup>-</sup> is indicated by stippled lines. Note the frameshift caused by exon 4 deletion (underlined grey residues). **d**, RNase protection with whole-brain RNA by mRNAs for cyclophilin (Cyclo) as internal standard, ADAR1, and ADAR2 in three wild-type (+/+) and three *ADAR2*-deficient (-/-) mice. Lane 1, unprotected radiolabelled antisense RNA probes for cyclophilin (324 nt), ADAR1 (283 nt) and ADAR2 (248 nt). Lanes 2–7, protected probe fragments for cyclophilin (244 nt), ADAR1 (203 nt, exon 6–7 sequences<sup>25</sup> encoding RNA-binding domains) and ADAR2 (168 nt, exons 2–3 sequences<sup>16</sup> for RNA-binding domains). A probe derived from ADAR2 exon 6–7 sequences<sup>16</sup> for part of the deaminase domain yielded the same reduction for *ADAR2*<sup>-</sup> allele expression (not shown).



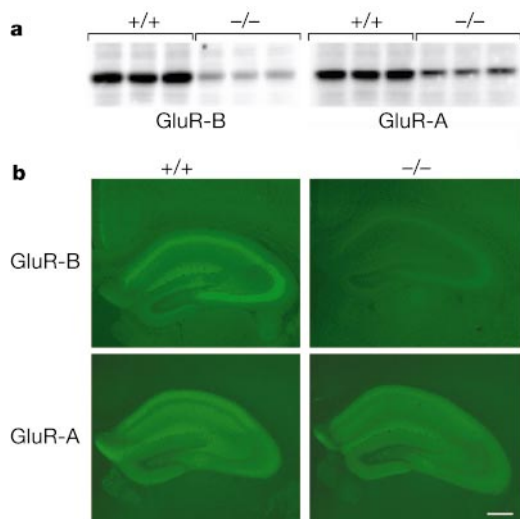
**Figure 2** GluR-B and GluR-A transcript analyses. **a**, RNase protection. Lane 1, unprotected radiolabelled antisense RNA probes for  $\beta$ -actin as internal standard (334 nt), GluR-B<sup>26</sup> (upper gel, 270 nt; lower gel, 272 nt) and GluR-A (221 nt); lanes 2–4, protection by whole-brain RNA from P14 *ADAR2*<sup>+/+</sup> mice (+/+); lanes 5–7, from P14 *ADAR2*<sup>-/-</sup> mice (-/-). Protected fragment sizes in nt:  $\beta$ -actin, 245; GluR-B, 190 (upper gel, exons 11–12) and 192 (lower gel, exons 3–4); GluR-A, 141 (exons 3–4). Note the reduction in

GluR-B mRNA in the mutant is only revealed with the exon 11–12 probe (upper gel), but not with the exon 3–4 probe (sum of pre- and mRNA; lower gel). **b**, *In situ* hybridization. Horizontal brain sections of *ADAR2*<sup>+/+</sup> and *ADAR2*<sup>-/-</sup> mice were hybridized with <sup>35</sup>S-labelled oligonucleotide probes<sup>24</sup> specific for GluR-B mRNA (36 nt, spanning exons 11–12) and pre-mRNA (37 nt, intron 11). Note the substantially higher levels of GluR-B pre-mRNA in the mutant and of GluR-B mRNA in wild type. Scale bar, 2 mm.

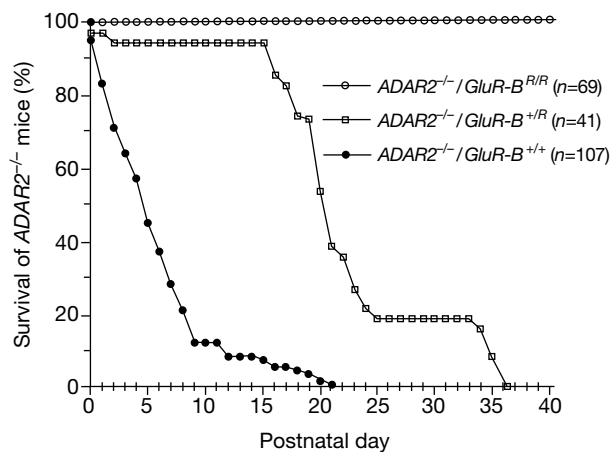
pyramidal cells and other principal neurones (cerebellar Purkinje cells and neocortical layer 5 pyramidal neurones) displayed, relative to wild type, pronounced rectification, increased desensitization rates and 30-fold higher Ca<sup>2+</sup> permeability ( $P_{Ca}/P_{Na}$  3.34–3.94 ( $n = 4–8$ ) compared with 0.12–0.14 ( $n = 4–10$ )). Moreover, the macroscopic AMPA receptor-mediated conductance in CA1 pyramidal cells of *ADAR2*<sup>-/-</sup> mice was significantly higher ( $40.8 \pm 4.0$  ( $n = 10$ ) compared with  $21.4 \pm 3.0 \mu S$  ( $n = 3$ ) in wild type) than in our mouse mutants expressing editing-deficient *GluR-B* genes<sup>11,12</sup>. As reported<sup>12</sup>, the increase in macroscopic AMPA conductance, caused by the higher single-channel conductance in absence of GluR-B(R) participation<sup>10</sup>, appears to be the primary cause for the high seizure susceptibility of young mice expressing Q/R site-unedited GluR-B transcripts.

To show that the prematurely lethal phenotype of *ADAR2*<sup>-/-</sup> mice

is engendered by insufficient Q/R site editing of GluR-B, we attempted a rescue of this phenotype by introducing a *GluR-B*<sup>R</sup> allele<sup>9</sup>. This allele, generated by targeting, contains an arginine (R) codon for the Q/R site, and its expression in *ADAR2*<sup>-/-</sup> mice is independent of Q/R site editing. We found that the severely compromised phenotype of *ADAR2*<sup>-/-</sup> mice was partially rescued already in heterozygous *ADAR2*<sup>-/-</sup>/*GluR-B*<sup>R/R</sup> mutants, which survived up to P35 (Fig. 4). Moreover, their phenotype was less impaired than that of *GluR-B*<sup>+/ $\Delta$ ECS</sup> mice, consistent with the hypothesis that phenotypic impairment increases with the Q/R ratio of GluR-B<sup>12</sup>. In both *ADAR2*<sup>-/-</sup>/*GluR-B*<sup>R/R</sup> and *GluR-B*<sup>+/ $\Delta$ ECS</sup> mutants, one GluR-B allele expresses exclusively GluR-B(R), but primary transcripts from the second allele are underedited and the corresponding mRNA levels are reduced. In mRNA derived from the *GluR-B* <sup>$\Delta$ ECS</sup> allele the Q/R site is not edited at all, whereas mRNA from the *GluR-B*<sup>R</sup> allele is Q/R site edited to 40% in the *ADAR2*<sup>-/-</sup> background. Thus, the Q/R ratio in GluR-B-containing AMPA receptors is lower in *ADAR2*<sup>-/-</sup>/*GluR-B*<sup>R/R</sup> than in *GluR-B*<sup>+/ $\Delta$ ECS</sup>



**Figure 3** GluR-B and GluR-A protein analyses. **a**, Western blots of brain protein from three *ADAR2*<sup>+/+</sup> (+/+) and three *ADAR2*<sup>-/-</sup> (-/-) mice with antibodies for two AMPA receptor subunits. **b**, Immunocytochemistry showing blow-ups of the hippocampus in coronal vibratome sections (40  $\mu m$ ) of P14 *ADAR2*<sup>+/+</sup> and *ADAR2*<sup>-/-</sup> brains, probed with antibodies to GluR-B (upper panels) and GluR-A (lower panels) and stained with FITC-conjugated secondary antibody. Note that a moderate GluR-A reduction is apparent in **a**, but barely detectable in **b**. Scale bar, 0.3 mm.



**Figure 4** Survival of *ADAR2*<sup>-/-</sup> mice. Data points depict the percentage of mice alive at the indicated postnatal days. The number of mice observed is listed in parentheses for each genotype. Note that the life span of *ADAR2*-deficient mice increased with the copy number of the sequence-modified *GluR-B*<sup>R</sup> allele, entirely consistent with the reduction in Q/R ratio, as elucidated by previous studies on the effect of Q/R site-uneditable *GluR-B* alleles in targeted mouse mutants<sup>11,12</sup>.

mice, and the phenotype is less affected. Full rescue of the impaired *ADAR2*<sup>-/-</sup> phenotype was achieved after introducing the second *GluR-B*<sup>R</sup> allele. *ADAR2*<sup>-/-</sup>/*GluR-B*<sup>R/R</sup> mice appeared normal at all ages, like *GluR-B*<sup>R/R</sup> mice<sup>9</sup>, as judged by food intake, weight increase, postnatal development, breeding and general behaviour. This identifies the *GluR-B* transcript as the most critical substrate for *ADAR2* in the mammal.

We investigated whether RNA editing at other known sites is affected by functional *ADAR2* ablation. We observed reduced editing at the majority of 25 positions in diverse transcripts (Table 1); even hemizygoty for *ADAR2* led at several sites to a modest decline in the extent of editing (Table 1). Notably, the actual reduction in the extent of editing may not have been revealed, as many of the sites were analysed at the mRNA rather than the pre-mRNA stage. Nevertheless, the results indicate a pleiotropic action of *ADAR2*, not entirely anticipated from *in vitro* studies<sup>14,21</sup>; *ADAR2* may act in concert with other RNA-editing enzymes, perhaps assembled with additional RNA interacting proteins in a multi-component nuclear 'editosome'. The reduced editing at many sites might also reflect, at least in part, interference with other *ADARs* by the residual truncated *ADAR2* protein (Fig. 1c); if so, affected editing levels might not indicate true catalysis by *ADAR2*. The severe reduction in transcript levels from the mutant allele (Fig. 1d), however, may render such interference unlikely.

In summary, we have shown that RNA editing can control the rate of splicing in nuclear transcripts; a link between editing and splicing has also been reported in *Drosophila*<sup>22</sup>. Moreover, our study identified the Q/R site codon in *GluR-B* pre-mRNA as a critical physiological substrate of *ADAR2*, apparently the only one at which editing is essential for viability, at least during the first few weeks of life. Whether or not dependence of viability on Q/R site editing extends into adulthood will be investigated by conditional *ADAR2* gene expression. As *ADAR2*<sup>-/-</sup> mice were phenotypically rescued by *GluR-B*<sup>R</sup> alleles, the effects caused by underediting of other *ADAR2* substrates need to be elucidated by future analyses of *ADAR2*<sup>-/-</sup>/*GluR-B*<sup>R/R</sup> mice. □

## Methods

### Extent of editing

RT-PCR products from brain RNA of the different genotypes were generated with primers specific for the pre-mRNAs or mRNAs (details of oligonucleotides are available from the author on request). Amplicons were cloned directionally into M13 RF DNA and more than 1,000 recombinant single-stranded phage plaques were hybridized to oligonucleotide probes that distinguish between edited and unedited positions in the different sequences<sup>6</sup>. Alternatively, amplicons were sequenced directly, and the approximate extent of editing was derived from the different peak heights for the two nucleotides occurring in identical positions in DNA sequence chromatograms.

### *GluR-B* mRNA levels by RT-PCR

RT-PCR was used with primers to co-amplify all four AMPA receptor subunit cDNAs<sup>23</sup>. Amplicons were cloned directionally, and filters containing more than 1,000 recombinant phage plaques were probed sequentially for the different subunit cDNAs. The numeric representations (in percent) of the four subunit sequences were for *ADAR2*<sup>-/-</sup> mice: *GluR-A*, 57; *GluR-B*, 14; *GluR-C*, 20; *GluR-D*, 9; for *ADAR2*<sup>+/-</sup> mice 36, 41, 15, 8, and for wild-type mice 39, 44, 11, 6.

### RNase protection

RNase protection (RPALIII Ribonuclease protection assay kit, Ambion) was used to determine transcripts levels for *GluR-A*, *GluR-B*, *ADAR1* and *ADAR2*. Individual reactions were performed with 20 µg total whole-brain RNA, and radiolabelled antisense RNA probes were as described in Fig. 2.

### *In situ* hybridization

*In situ* hybridization was performed on horizontal cryostat sections of P14 mouse brains with 3'-end-labelled oligonucleotides<sup>24</sup>. Exposure to X-ray film was for 7 days. Control sections hybridized with labelled probe in presence of a 100-fold excess of the unlabelled oligonucleotide generated no signal.

### Western analysis and immunocytochemistry

Western blots with 10 µg of P14 whole-brain protein extracts per lane were successively

probed, after intermittent stripping, with antibodies to *GluR-B* (1:200; Pharmingen) and *GluR-A* (1:300; Chemicon); secondary antibody was conjugated to horseradish peroxidase (1:40,000; Amersham) and detection was with Super Signal West (Pierce). P14 brain sections were processed for immunocytochemistry<sup>19</sup>, incubated with antibodies to *GluR-B* (1:10) and *GluR-A* (1:50) and stained with FITC-conjugated goat anti-rabbit IgG (1:100; Jackson ImmunoResearch).

## Electrophysiology

AMPA receptor-mediated currents in whole-soma 'nucleated' patches of identified neurones in acute brain slices of P14 mice were elicited and analysed as described<sup>12</sup>.

Received 10 March; accepted 22 May 2000.

- Bass, B. L. RNA editing. An I for editing. *Curr. Biol.* **5**, 598–600 (1995).
- Rueter, S. & Emeson, R. in *Modification and Editing of RNA* (eds Grosjean, H. & Benne, R.) 343–361 (ASM, Washington DC, 1998).
- Seeburg, P. H., Higuchi, M. & Sprengel, R. RNA editing of brain glutamate receptor channels: mechanism and physiology. *Brain Res. Rev.* **26**, 217–229 (1998).
- Burns, C. M. *et al.* Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature* **387**, 303–308 (1997).
- Rueter, S. M., Dawson, T. R. & Emeson, R. B. Regulation of alternative splicing by RNA editing. *Nature* **399**, 75–79 (1999).
- Higuchi, M. *et al.* RNA editing of AMPA receptor subunit *GluR-B*: A base-paired intron–exon structure determines position and efficiency. *Cell* **75**, 1361–1370 (1993).
- Melcher, T. *et al.* A mammalian RNA editing enzyme. *Nature* **379**, 460–464 (1996).
- Bass, B. L. *et al.* A standardized nomenclature for adenosine deaminases that act on RNA. *RNA* **3**, 947–949 (1997).
- Kask, K. *et al.* The AMPA receptor subunit *GluR-B* in its Q/R site-unedited form is not essential for brain development and function. *Proc. Natl Acad. Sci. USA* **95**, 13777–13782 (1998).
- Dingledine, R., Borges, K., Bowie, D. & Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **51**, 7–61 (1999).
- Brusa, R. *et al.* Early-onset epilepsy and postnatal lethality associated with an editing-deficient *GluR-B* allele in mice. *Science* **270**, 1677–1680 (1995).
- Feldmeyer, D. *et al.* Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit *GluR-B*. *Nature Neurosci.* **2**, 57–64 (1999).
- Melcher, T. *et al.* RED2, a brain-specific member of the RNA-specific adenosine deaminase family. *J. Biol. Chem.* **271**, 31795–31798 (1996).
- Maas, S. *et al.* Different structural and enzymatic requirements for RNA editing in glutamate receptor pre-mRNAs. *J. Biol. Chem.* **271**, 12221–12226 (1996).
- Lai, F., Chen, C. X., Carter, K. C. & Nishikura, K. Editing of glutamate receptor B subunit ion channel RNAs by four alternatively spliced DRADA2 double-stranded RNA adenosine deaminases. *Mol. Cell. Biol.* **17**, 2413–2424 (1997).
- Villard, L., Tassone, F., Haymowicz, M., Welborn, R. & Gardiner, K. Map location, genomic organization and expression patterns of the human RED1 RNA editase. *Somat. Cell. Mol. Gen.* **23**, 135–145 (1997).
- Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W. & Roder, J. C. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc. Natl Acad. Sci. USA* **90**, 8424–8428 (1993).
- Dabiri, G. A., Lai, F., Drakas, R. A. & Nishikura, K. Editing of *GluR-B* ion channel RNA in vitro by recombinant double-stranded RNA adenosine deaminase. *EMBO J.* **15**, 34–45 (1996).
- Zamanillo, D. *et al.* Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* **284**, 1805–1811 (1999).
- Jia, Z. *et al.* Enhanced LTP in mice deficient in the AMPA receptor *GluR2*. *Neuron* **17**, 945–956 (1996).
- Herb, A., Higuchi, M., Sprengel, R. & Seeburg, P. H. Q/R site editing in kainate receptor *GluR5* and *GluR6* pre-mRNAs requires distant intronic sequences. *Proc. Natl Acad. Sci. USA* **93**, 1875–880 (1996).
- Reenan, R. A., Hanrahan, C. J. & Ganetzky, B. The *mlepnaps* RNA helicase mutation in *Drosophila* results in a splicing catastrophe of the *para Na*<sup>+</sup> channel transcript in a region of RNA editing. *Neuron* **25**, 139–149 (2000).
- Geiger, J. R. P. *et al.* Relative abundance of subunit mRNAs determines gating and Ca<sup>2+</sup> permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* **15**, 193–204 (1995).
- Wisden, W. & Morris, B. J. in *In Situ Protocols for the Brain* (eds Wisden, W. & Morris, B. J. 9–30 (Academic, London, 1994).
- Wang, Y., Zeng, Y., Murray, J. M. & Nishikura, K. Genomic organization and chromosomal localization of the human dsRNA adenosine deaminase gene: the enzyme for glutamate-activated ion channel RNA editing. *J. Mol. Biol.* **254**, 184–195 (1995).
- Köhler, M., Kornau, H. -C. & Seeburg, P. H. The gene for the principal AMPA receptor subunit *GluR-B*: organization and sequences for alternatively spliced and edited transcripts. *J. Biol. Chem.* **269**, 17367–17370 (1994).
- Lomeli, H. *et al.* Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. *Science* **266**, 1709–1713 (1994).

## Acknowledgements

We thank R. Wenthold for the antibody to *GluR-B*; A. Nagy for the murine embryonic stem cell line R1; K. Kask for help with *GluR-B*<sup>R</sup> mice; F. Zimmermann for blastocyst injection; S. Grünwald and H. Grosskurth for DNA sequencing; U. Amtmann for *in situ* hybridization; and H. Avci, C. Faul and C. Baust for technical help. This work was supported, in part, by the Deutsche Forschungsgemeinschaft, the Human Frontier Science Program and the Bristol-Myers Squibb foundation.

Correspondence and requests for materials should be addressed to P.H.S. (e-mail: seeburg@mpimf-heidelberg.mpg.de).