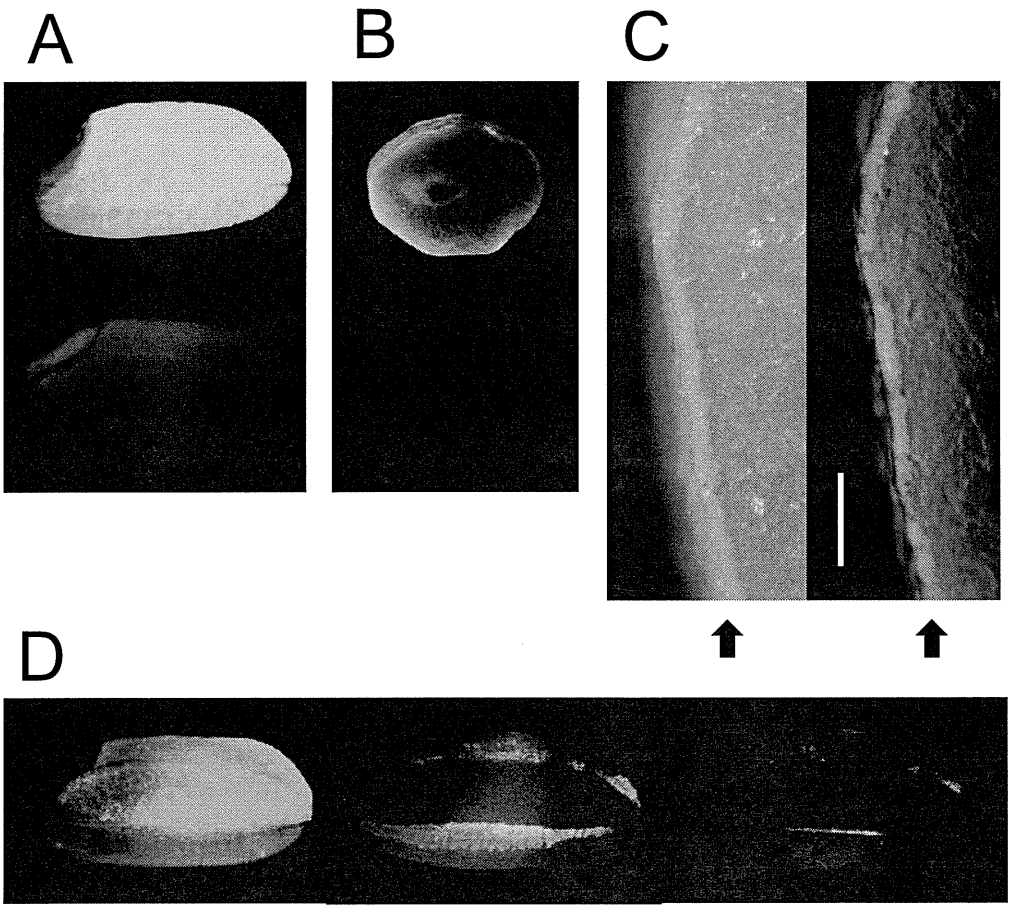


Fig. 1. Detection of GFP fluorescence in seeds (brown rice). (A) Transgenic brown rice (upper seed) and non-transgenic brown rice (lower seed). (B) Transverse sections of transgenic brown rice (upper seed) and non-transgenic brown rice (lower seed). (C) Aleurone layer in transverse section of transgenic brown rice. Right panel shows fluorescence view. Bar indicates 200 μm. Arrows show aleurone layer. (D) Effects of polishing transgenic brown rice: non-polished rice (left seed, weight: 21.3 mg), the roughly polished rice (middle seed), and the polished rice (right seed, weight: 19.1 mg, rice-polishing rate: 89.3%). The yellow region of roughly polished rice is bran layer. A fluorescence stereo-microscope (Nikon SMZ800) was used to observe GFP fluorescence.

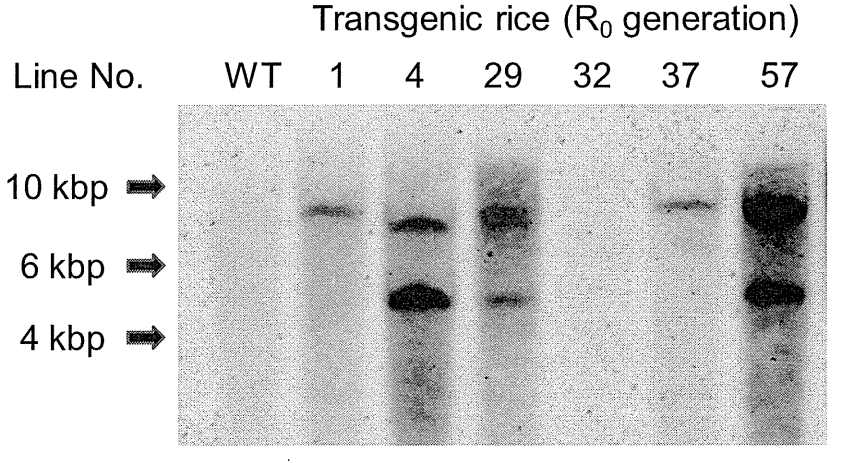


Fig. 2. Southern blot analysis of Xba I-digested total DNA probed for GFP-specific genes. WT, non-transgenic rice plant: R_0, primary transgenic rice plant.

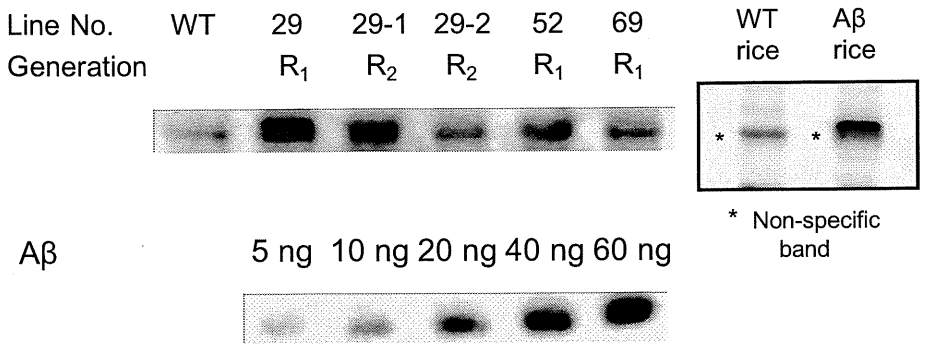


Fig. 3. Determination of Aβ42 expression levels. Brown rice samples were subjected to SDS-PAGE with Aβ42 at increasing concentration (5, 10, 20, 40 and 60 ng). Approximately 0.15 mg of crushed seeds was applied to each lane. R_0 seeds (R_1 generation) and R_1 seeds (R_2 generation) were used. WT; non-transgenic rice; R_1, R_0 progeny; R_2, R_1 progeny. Faint band in WT is non-specific band just below Aβ42 band.

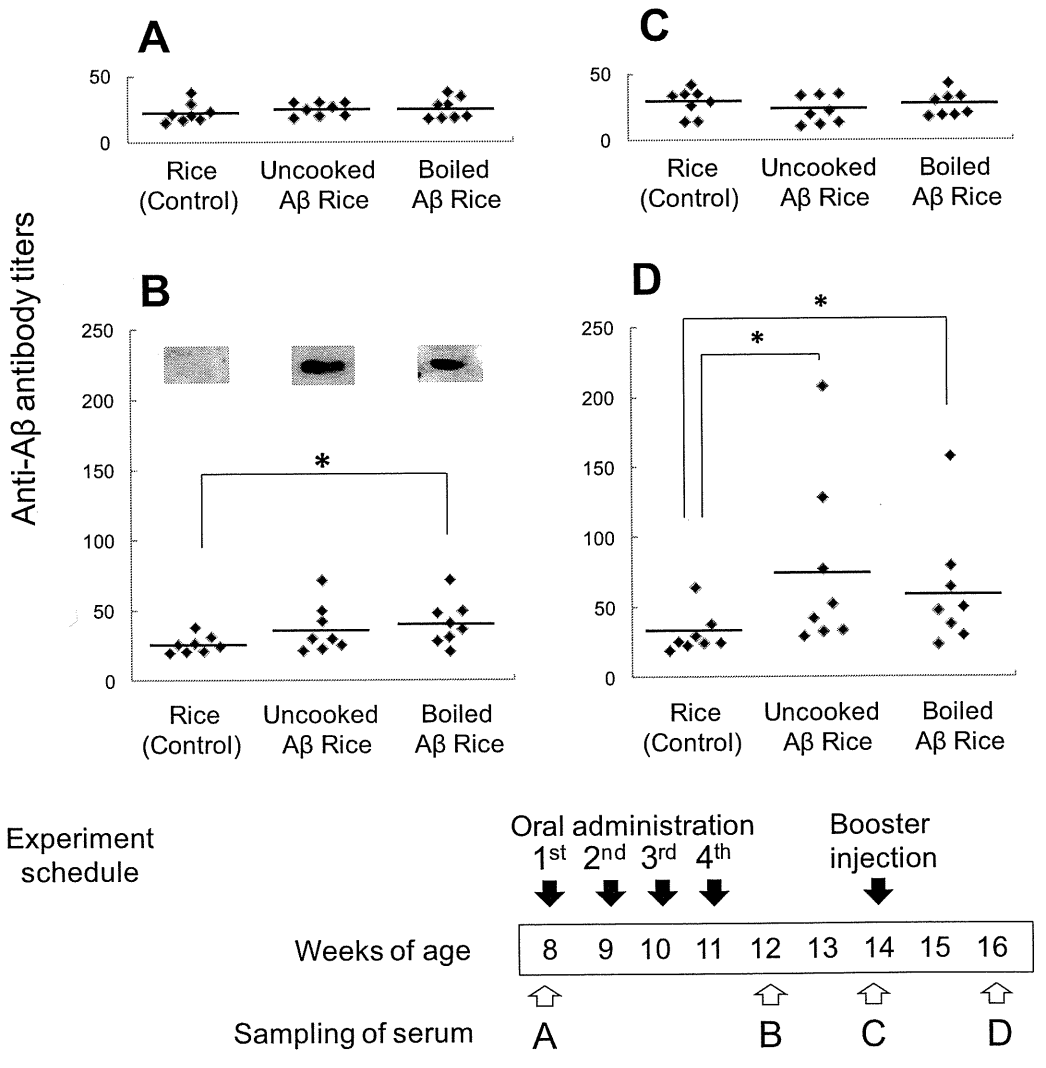


Fig. 4. Titers of antibodies against Aβ in serum from 8-week-old mice before immunization (A), 12-week-old and 14-week-old mice after immunization (B, C, each), and 16-week-old mice after booster injection (D). Anti-Aβ antibody titers for each mouse are shown. Horizontal lines show average.* $P < 0.05$ vs. control. Data were compared by *t*-test after logarithmic conversion. Antibody production evaluated by Western blot analysis (B). For each group, four serum samples with highest anti-Aβ antibody titers were mixed and used for Western blot analysis.

The increase of anti-Aβ antibody titer after booster injection shows the presence of anti-Aβ antibody response in mice fed uncooked Aβ rice or boiled Aβ rice. However, the increase in anti-Aβ antibody titer at 12 weeks of age shows the booster injection was not necessary for Aβ oral immunization.

In a previous study using green pepper containing Aβ [11], we examined the effect on anti-Aβ antibody titers of orally immunized mice and subcutaneously immunized mice over a long-term (12.5 months) trial. The increases in anti-Aβ antibody titer of orally immunized mice were similar to increases in injected mice. In the present study, the increases in anti-Aβ antibody titers of mice fed Aβ rice were not as great as those observed in mice given a booster injection. The difference between the studies may arise from the differences in the period of antigen administration. Further, even if an antibody response is weak, Aβ in mice brains may be removed by long-term immunization of the AD mouse models [21]. Taken together, we conclude that long-term oral administration of Aβ rice without Aβ injection can prevent and treat AD in mice.

In general, the immunological effect after oral-intestinal mucosal immunization tends to be weak, and this method may induce immunological tolerance. Oral immunological tolerance can be suppressed by the use of specific adjuvants. Bacterial toxin, such as CTB, is often used as an adjuvant in oral immunization of mice. Although CTB may not be highly toxic, there may be some clinical side effects. A safer adjuvant might be developed from plants that produce compounds such as saponin [22] and it may be feasible to develop adjuvant-free oral vaccine from plants. Further animal study is necessary to determine the effectiveness of adjuvant-free Aβ rice for AD.

In a previous study, we developed a technique in which a plant (green pepper) was infected with a plant virus, causing Aβ to accumulate within the plant [12]. Mice that were orally administered Aβ-containing plant tissue showed lower levels of serum IgG2a, an inflammatory Th1 immunological globulin, than mice in which the vaccine was administered by injection [11]. These results indicate that the plant-derived vaccine is safe and effective. In addition, vaccines made using plants are far safer than vaccines from animal cells or microbes as there is less danger of the vaccine being adulterated with prion proteins, pathogenic viruses, or bacterial toxins. Thus, plant-derived vaccines require less purification, and may be produced cheaply.

The rice cultivar 'Hayayuki' used in this study is an early-ripening variety that can be harvested approximately 3 months after planting. Moreover, its compact form allows year-round production in a greenhouse or plant factory so that transgenic rice would be easily contained. The additional cost of contained production is likely to be justified by a high added-value product, such as a remedy for AD.

In the present study, we showed that oral administration of Aβ rice to mice elevated serum Anti-Aβ antibody titer. We previously found oral administration of Aβ green pepper to Tg2576 mouse models elevated serum Anti-Aβ antibody titer and reduced senile plaques; and that there was an inverse correlation between anti-Aβ antibody titers and soluble intracerebral Aβ [11]. It is likely that accumulation of Aβ in the brain can be suppressed by administering Aβ rice. We plan a further experiment with AD mouse models to investigate whether oral immunization by long-term administration of Aβ rice decreases senile plaques.

Rice is commonly eaten in grain form without first being pulverized. This would make it easy to control intake, as with medicines in pill form. In addition, where rice is eaten as a staple, it is possible to ensure regular intake. In the present study, we showed that boiled Aβ rice does not reduce the efficacy of the vaccine, thereby allowing its use as an edible vaccine. The ease of use of an Aβ rice vaccine for AD makes this the most attractive vaccine for preventing and treating the disease.

Acknowledgements

We would like to thank Dr. S. Hidaka and Dr. N. Yamagishi for valuable advice. This work was supported in part by funding for Research for Promoting Technological Seeds from the Japan Science and Technology Agency.

Conflict of interests

The authors have declared that no conflict of interest exists.

References

1. Selkoe DJ. Alzheimer's disease: a central role for amyloid. J Neuropathol Exp Neurol. 1994; 53: 438-447.
2. Carlson GA. A welcoming environment for amyloid plaques. Nat Neurosci. 2003; 6: 328-330.
3. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999; 400: 173-177.
4. Bard F, Cannon C, Barbour R, et al. Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med. 2000; 6: 916-919.
5. Weiner HL, Lemere CA, Maron R, et al. Nasal administration of amyloid-β peptide decreases cerebral amyloid burden in a

mouse model of Alzheimer's disease. Ann Neurol. 2000; 48: 567-579.
6. Janus C, Pearson J, McLaurin J, et al. Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000; 408: 979-982.
7. Morgan D, Diamond DM, Gottschall PE, et al. Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000; 408: 982-985.
8. Check E. Nerve inflammation halts trial for Alzheimer's drug. Nature 2002; 415: 462.
9. Holmes C, Boche D, Wilkinson D, et al. Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 2008; 372: 216-223.
10. Kim HD, Cao Y, Kong K, et al. Induction of a Th2 immune response by co-administration of recombinant adenovirus vectors encoding amyloid β-protein and GM-CSF. Vaccine 2005; 23: 2977-2986.
11. Ishii-Katsuno R, Nakajima A, Katsuno T, et al. Reduction of amyloid β-peptide accumulation in Tg2576 transgenic mice by oral vaccination. Biochem Biophys Res Commun. 2010; 399: 593-599.
12. Szabó B, Hori K, Nakajima A, et al. Expression of amyloid-β 1-40 and 1-42 peptides in Capsicum annum var. angulosum for oral immunization. Assay Drug Dev Technol. 2004; 2: 383-388.
13. Kim HS, Euym JW, Kim MS, et al. Expression of human β-amyloid peptide in transgenic potato. Plant Sci. 2003; 165: 1445-1451.
14. Youm JW, Kim H, Han JH, et al. Transgenic potato expressing Aβ reduce Aβ burden in Alzheimer's disease mouse model. FEBS Lett. 2005; 579: 6737-6744.
15. Chu CC, Wang CC, Sun CS, et al. Establishment of an efficient medium for another culture of rice through comparative experiments on the nitrogen sources. Sci Sin. 1975; 18: 659-668.
16. Toki S, Hara N, Ono K, et al. Early infection of scutellum tissue with Agrobacterium allows high-speed transformation of rice. Plant J. 2006; 47: 969-976.
17. Niwa Y, Hirano T, Yoshimoto K, et al. Non-invasive quantitative detection and applications of non-toxic, S65T-type green fluorescent protein in living plants. Plant J. 1999; 18: 455-463.
18. Ohta S, Mita S, Hattori T, et al. Construction and expression in tobacco of a β-glucuronidase (GUS) reporter gene containing an intron within the coding sequence. Plant Cell Physiol. 1990; 31: 805-813.
19. Hiei Y, Ohta S., Komari T, et al. Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J. 1994; 6: 271-282.
20. Rashid H, Yokoi S, Toriyama K, et al. Transgenic plant production mediated by Agrobacterium in Indica rice. Plant Cell Rep. 1996; 15: 727-730.
21. Sigurdsson EM, Knudsen E, Asuni A, et al. An attenuated immune response is sufficient to enhance cognition in an Alzheimer's disease mouse model immunized with amyloid-beta derivatives. J Neurosci. 2004; 24: 6277-6282.
22. Pickering RJ, Smith SD Strugnell RA, et al. Crude saponins improve the immune response to an oral plant-made measles vaccine. Vaccine 2006; 24: 144-150.

Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2011 | 116 | 76–81 doi: 10.1111/j.1471-4159.2010.07086.x

Polypyrimidine tract-binding protein 1 regulates the alternative splicing of dopamine receptor D_2

Toshikazu Sasabe,*,† Eugene Futai* and Shoichi Ishiura*

*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

†Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists

Abstract

Dopamine receptor D_2 (DRD2) has two splicing isoforms, a long form (D2L) and short form (D2S), which have distinct functions in the dopaminergic system. However, the regulatory mechanism of the alternative splicing of *DRD2* is unknown. In this study, we examined which splicing factors regulate the expression of D2L and D2S by over-expressing several RNA-binding proteins in HEK293 cells. In a cellular splicing assay, the over-expression of polypyrimidine tract-binding protein 1 (PTBP1) reduced the expression of D2S, whereas the knockdown of PTBP1 increased the expression of D2S. We also identified the regions of *DRD2* that are responsive to PTBP1 using heterologous minigenes and deletion mutants. Our results indicate that PTBP1 regulates the alternative splicing of *DRD2*. Considering that DRD2 inhibits cAMP-dependent protein kinase A, which modulates the intracellular localization of PTBP1, PTBP1 may contribute to the autoregulation of DRD2 by regulating the expression of its isoforms.

Keywords: alternative splicing, dopamine, dopamine receptor D_2, PTBP1.

J. Neurochem. (2011) **116**, 76–81.

Dopamine is the predominant neurotransmitter in the CNS, where it plays a leading role in the regulation of such physiological functions as locomotor activity, cognition, positive reinforcement, and hormone secretion. The effects of dopamine are mediated by its binding to five G-protein-coupled receptors, which are divided into two subclasses: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). Dopamine receptor D_2 (DRD2) is the main autoreceptor of the dopaminergic system (Centonze *et al.* 2002); however, it is also critical for post-synaptic transmission (Usiello *et al.* 2000).

Alternative gene splicing generates two distinct isoforms of DRD2, a long form (D2L) and short form (D2S), which differ in the presence of a 29-amino-acid insert in the third cytoplasmic loop. D2L is expressed mainly in post-synaptic regions, whereas D2S is expressed mainly in pre-synaptic regions (Khan *et al.* 1998; Usiello *et al.* 2000). These isoforms differentially contribute to the pre-synaptic inhibition of glutamate and GABA transmission (Centonze *et al.* 2004); moreover, they exhibit specific G_i protein preferences (Senogles 1994; Guiramand *et al.* 1995; Senogles *et al.* 2004) and have distinct roles in the regulation of protein phosphorylation (Lindgren *et al.* 2003). Furthermore, behavioral studies of D2L-deficient mice have shown that D2L and D2S contribute differentially to the regulation of certain motor functions (Usiello *et al.* 2000; Wang *et al.* 2000) and emotional responses (Hranilovic *et al.* 2008). Similarly, human genetic studies have shown that the intronic single nucleotide polymorphism rs1076560, which has a significant effect on the expression ratio of the DRD2 isoforms, is associated with cognitive processing (Zhang *et al.* 2007) and emotional processing (Blasi *et al.* 2009). These results suggest that the expression ratio of the DRD2 isoforms is important for their functions.

However, little is known about the regulatory mechanism that mediates the alternative splicing of *DRD2*. Although it has been reported that haloperidol, sex steroid hormones, and ethanol affect the expression of splice variants (Arnauld *et al.* 1991; Guivarc'h *et al.* 1995, 1998; Oomizu *et al.* 2003), the molecular basis for these differences is unclear. In general, changes in splicing patterns are directed by regula-

Received August 26, 2010; revised manuscript received October 18, 2010; accepted October 22, 2010.

Address correspondence and reprint requests to Dr Shoichi Ishiura. Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo. 3-8-1, Komaba, Meguro-ku, Tokyo 153-8902, Japan. E-mail: cishiura@mail.ecc.u-tokyo.ac.jp

Abbreviations used: D2L, long form of DRD2; D2S, short form of DRD2; DRD2, dopamine receptor D_2; nPTB, neural PTB; PTBP1, polypyrimidine tract-binding protein 1; Tpm2, tropomyosin 2.

tory proteins that bind the pre-mRNA sequence and enhance or silence particular splicing choices (Li *et al.* 2007). Thus, in this study, we searched for proteins that regulate the alternative splicing of *DRD2* using a cellular splicing assay and identified the involvement of the splicing factor polypyrimidine tract-binding protein 1 (PTBP1).

Materials and methods

Plasmid construction

The region from exon 5 to exon 7 of *DRD2* was amplified from human genomic DNA and cloned into the *Xho*I-*Hind*III site of pEYFP-C1 (Clontech, Mountain View, CA, USA) (Fig. 1a). The open reading frames that encode SF2/ASF, PTBP1, nPTB, NOVA1, HuB, FOX2, hnRNP A1, and Tra2b were amplified by PCR from a human fetal brain cDNA library (Clontech) and cloned into pcDNA3.1/V5-His (Invitrogen, Carlsbad, CA, USA) using conventional biological techniques. Primer sequences are listed in Table S1. Plasmid constructions of NAPOR and FOX1 are gifts from Dr. Yoshihiro Kino, RIKEN Brain Science Institute, and hnRNP H from Dr. Kinji Ohno, Nagoya University. Heterologous minigenes were generated by inserting *DRD2* fragments containing exon 6, exon 7 and flanking regions into pEGFP-Tpm2-ex1-2 (a gift from Dr. Kino, RIKEN Brain Science Institute). *DRD2* deletion mutants were generated by inverse PCR from the wild-type plasmid using primers flanking the deleted regions. The nucleotide sequences of the DNA inserts were confirmed by sequencing.

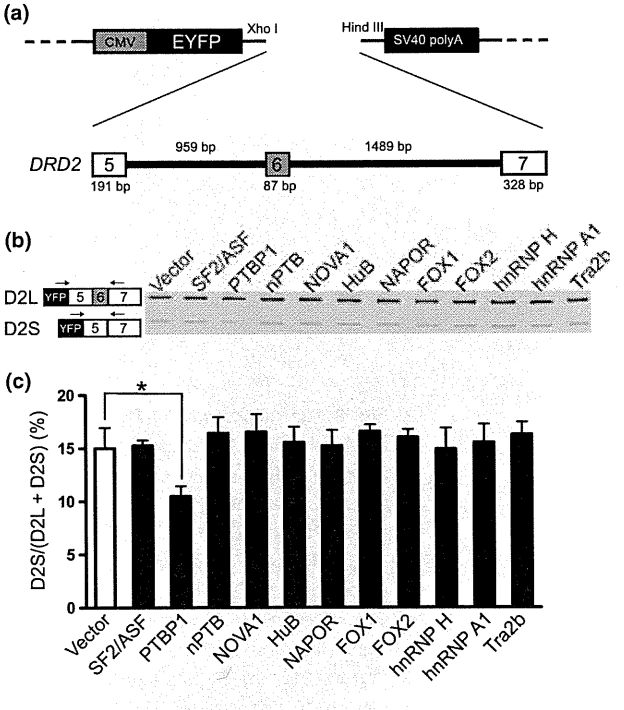


Fig. 1 The over-expression of PTBP1 reduced the alternative splicing of D2S. (a) Structure of the *DRD2* minigene. (b) Representative result from RT-PCR assays in which the *DRD2* minigene and plasmids for expressing RNA-binding proteins were transfected into HEK293 cells. The upper bands correspond to the splice product containing exon 6 (D2L), while the lower bands correspond to the splice product lacking exon 6 (D2S). (c) Bar chart showing the quantified percentage of D2S (Mean + SEM, $n = 3$). The statistical significance was analyzed by Dunnett's multiple-comparison test (*$p < 0.05$).

Cell culture and transfection

HEK293 and SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and incubated at 37°C with 5% CO_2. For the minigene assays, HEK293 cells were transfected with plasmids for the expression of minigene and V5-tagged proteins using Fugene 6 (Roche Diagnostics, Basel, Switzerland). In our RNAi experiments, HEK293 cells were transfected with the minigene plasmids and an siRNA for *PTBP1* (Invitrogen, Stealth™ Select RNAi HSS143520, and Negative Control Hi GC) and *nPTB* (Invitrogen, Stealth™ Select RNAi HSS126818, and Negative Control Lo GC) using Lipofectamine 2000 (Invitrogen), and SH-SY5Y cells were transfected with the siRNA using Lipofectamine RNAiMAX (Invitrogen) and the Reverse Transfection protocol. The efficacy of the RNAi-mediated knockdown of endogenous PTBP1, nPTB, and actin expressions was determined by western blot analysis using anti-PTBP1 (Invitrogen, catalog No. 32-4800), anti-nPTB (Abnova, Taipei City, Taiwan, catalog No. H00058155-A01), and anti-actin (Sigma-Aldrich, St. Louis, MO, USA, catalog No. A2066) antibodies.

Identification of DRD2 splice variants

Forty-eight hours after transfection, total RNA was isolated from the cells using a GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich). cDNA synthesis was performed using a PrimeScript First Strand cDNA Synthesis Kit (TAKARA BIO, Shiga, Japan) using oligo dT primer. The DRD2 minigene fragments were amplified by PCR (20 cycles) using a forward primer specific for the 3′ region of *EYFP* (AAGTCCGGACTCAGATCTCG) and a *DRD2*-specific reverse primer (DRD2-Ex7-Rv) that annealed to the 5′ region of exon 7 (CATCTCCATCTCCAGCTCCT). To detect endogenous *DRD2* fragments, a forward primer specific for exons 4 and 5 (CAATAACGCAGACCAGAACG) and DRD2-Ex7-Rv were used (40 cycles). For tropomyosin 2 (Tpm2)-based minigenes, primers green fluorescence protein (GFP)-Fw (CATGGTCCTGCTGGAGTTCGTG) and Tpm2-ex2-splicing-Rv2 (GGAGGGCCTGCTGCTCTTC) were used (Kino *et al.* 2009). The amplified products were resolved by 6% polyacrylamide gel electrophoresis and visualized using ethidium bromide. The intensities of the bands corresponding to the long and short forms were quantified by LAS-3000 and MultiGage software (Fuji Film, Tokyo, Japan). The quantified values were divided by the number of base pairs.

Results

PTBP1 regulates the alternative splicing of DRD2

To identify trans-acting factors that regulate the alternative splicing of *DRD2*, we used RT-PCR to detect splice variants. We constructed a gene fragment encompassing exons 5 through 7 of human *DRD2* in the vector pEYFP (Fig. 1a). This minigene was then transfected into HEK293 cells, and the expression ratios of D2L and D2S were analyzed by

RT-PCR. When the *DRD2* minigene was transfected with empty pcDNA3.1, the percentage of D2S was about 15% (Fig. 1b and c). Next, we expressed V5-tagged versions of several proteins known to regulate pre-mRNA splicing in the nervous system (SF2/ASF, PTBP1, nPTB, NOVA1, HuB, NAPOR, FOX1, FOX2, hnRNP H, hnRNP A1, and Tra2b); notably, SF2/ASF was previously proposed to regulate the alternative splicing of *DRD2* (Oomizu *et al.* 2003). Among the proteins tested, only when PTBP1 was transfected with the *DRD2* minigene was the percentage of D2S significantly reduced (to about 10%; Fig. 1b and c). We have confirmed the expressions of each RNA-binding proteins by western blot analysis and noted that the abundance of nPTB, NAPOR, and FOX1 are low (Figure S1). In addition, we showed the effects of PTBP1 were concentration dependent (Figure S2).

Next, we knocked down endogenous PTBP1 expression using an siRNA to confirm the effect of PTBP1 on *DRD2* splicing. We first confirmed the efficacy of the siRNA in modulating the expression of the target protein by western blot analysis (Fig. 2b). The presence of two PTBP1 bands rather than one is most likely because of phosphorylation. (Grossman *et al.* 1998). When the *DRD2* minigene was transfected with an siRNA for *PTBP1*, the percentage of D2S was significantly increased compared to transfection with a control siRNA (Fig. 2a). We also examined the effect of the knockdown of nPTB, a homologue of PTBP1, because it was reported that appearance of some exons are affected by both PTBP1 and nPTB (Boutz *et al.* 2007). The knockdown of PTBP1 increased the expression of nPTB (Fig. 2b), consistent with the previous reports (Boutz *et al.* 2007; Makeyev *et al.* 2007). While endogenous nPTB level was remarkably low and the knockdown of nPTB by siRNA was not observed, the increase in nPTB expression by the knockdown of PTBP1 was clearly inhibited by a siRNA for nPTB (Fig. 2b). Even when the increase in nPTB was inhibited, the knockdown of PTBP1 still increased the production of D2S splice variant (Fig. 2a), suggesting that the increase in nPTB has little or no effect on the alternative splicing of DRD2. Furthermore, we examined whether PTBP1 regulates the alternative splicing of endogenous *DRD2* in human neuroblastoma SH-SY5Y cells. When the siRNA for *PTBP1* was transfected into SH-SY5Y cells, the percentage of endogenous D2S fragments was also increased (Fig. 2c and d).

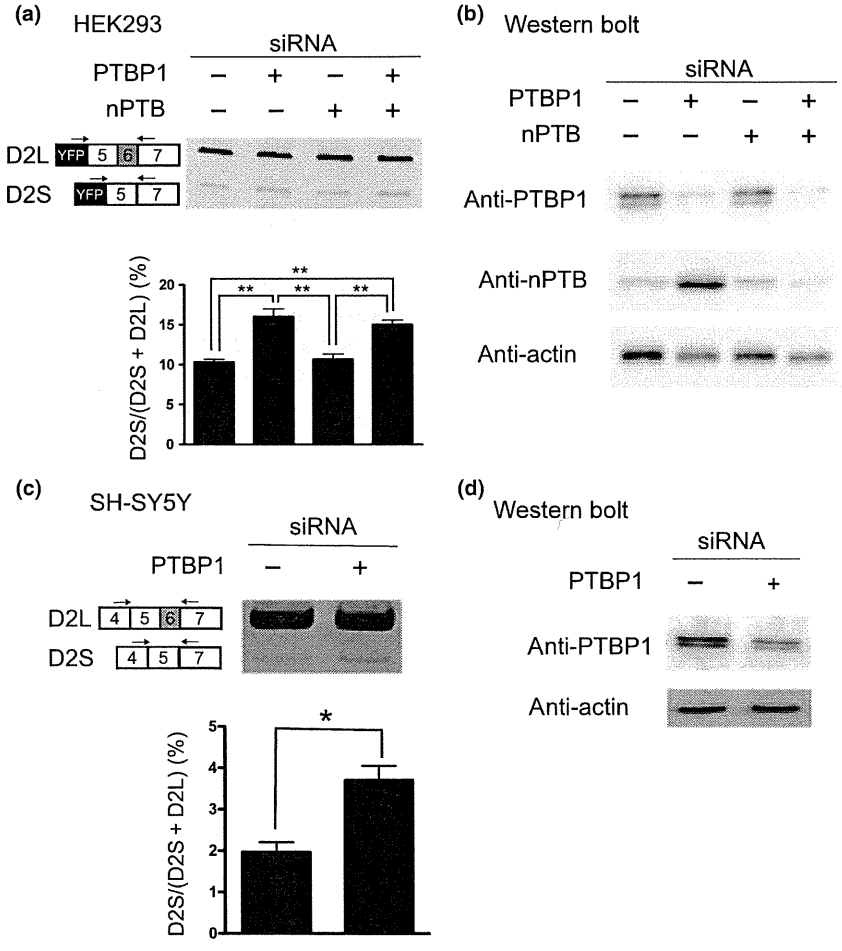


Fig. 2 The knockdown of PTBP1 increased the production of D2S splice variant. (a) Representative result from our cellular splicing assay using the *DRD2* minigene and siRNA for *PTBP1* and *nPTB* in HEK293 cells. Bar charts show the quantified percentages of D2S (Mean + SEM, $n = 3$). The statistical significances were analyzed using Tukey's multiple comparison test (**$p < 0.01$). (b) Representative result of western blot analysis of PTBP1 and nPTB in HEK293 cells. (c) Representative result of endogenous *DRD2* splicing using a siRNA for *PTBP1* in SH-SY5Y cells. Bar charts show the quantified percentages of D2S (Mean + SEM, $n = 3$). The statistical significance was analyzed using *t*-tests (*$p < 0.05$). (d) Representative result of western blot analysis of PTBP1 in SH-SY5Y cells.

Intronic regions flanking exon 6 are required for the PTBP1-mediated regulation of DRD2 splicing

To define the regions of *DRD2* that are required for its regulation by PTBP1, we utilized several previously generated heterologous minigenes (Kino *et al.* 2009). In these minigenes, the regions of interest were inserted in the context of constitutive exons of mouse *Tpm2*, which is distinct from *DRD2*. A reference fragment containing exon 9 of *Tpm2* and its flanking intronic regions or a *DRD2* fragment containing exon 6 or exon 7 and their flanking regions were inserted into a *Tpm2* fragment covering exons 1 and 2 (Fig. 3a). First, we predicted branch sites by a web-based program called ESEfinder 3.0 (Table S2, http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi). Then, using our *Tpm2*-based heterologous minigenes, we found that 14 bp upstream and 5 bp downstream of exon 6 are necessary for proper splicing (Fig. 3b). When exon 7 of DRD2 was inserted into Tpm2 cassette, a shorter exon 7 lacking the first 140 nucleotides was the main product. It was shown that 17 bp upstream of exon 7 is necessary for the splicing of full-length exon 7 (Fig. 3c). Because the primary elements regulating alternative splicing are thought to be located up to 200–300 nucleotides upstream and/or downstream of the regulated exon (Cooper 2005), a *DRD2* fragment stretching from 200 bp upstream of exon 6 (760 bp downstream of exon 5) to 200 bp downstream of exon 6 was used to examine the binding sequence of PTBP1 (Fig. 4a). PTBP1 had no effect on the inclusion of *Tpm2* exon 9 in HEK293 cells (Fig. 4b, left). In contrast, PTBP1 repressed *DRD2* exon 6 inclusion of the heterologous minigene, demonstrating that the inserted fragment of *DRD2* was sufficient for the response to PTBP1 (Fig. 4b, right). Next, to examine which region is necessary for the gene's responsiveness to PTBP1, we constructed *DRD2* deletion mutants lacking 200 bp upstream of exon 6 (Δint5_760–945), downstream of exon 6 (Δint6_6–200) or upstream of exon 7 (Δint6_1290–1487) (Fig. 4c). These deletion mutants were designed to include the regions that are necessary for splicing of exon 6 and exon 7. As shown in Fig. 4(d), Δint5_760–945 and Δint6_6–200 mutations altered the basal splicing pattern. Both deletion mutants exhibited markedly increased exclusion of exon 6 (from 15% to about 60% with vector transfection), suggesting the presence of elements in the deleted regions that enhance the inclusion of exon 6. Further, the over-expression of PTBP1 had no effect on either deletion mutant, indicating that both mutants had impaired responsiveness to PTBP1 (Fig. 4d). On the other hand, the over-expression of PTBP1 reduced D2S in the Δint6_1290–1487 mutant as well as a wild-type minigene, suggesting that PTBP1 affects the alternative splicing of DRD2 in regions other than the 3′ end of intron 6.

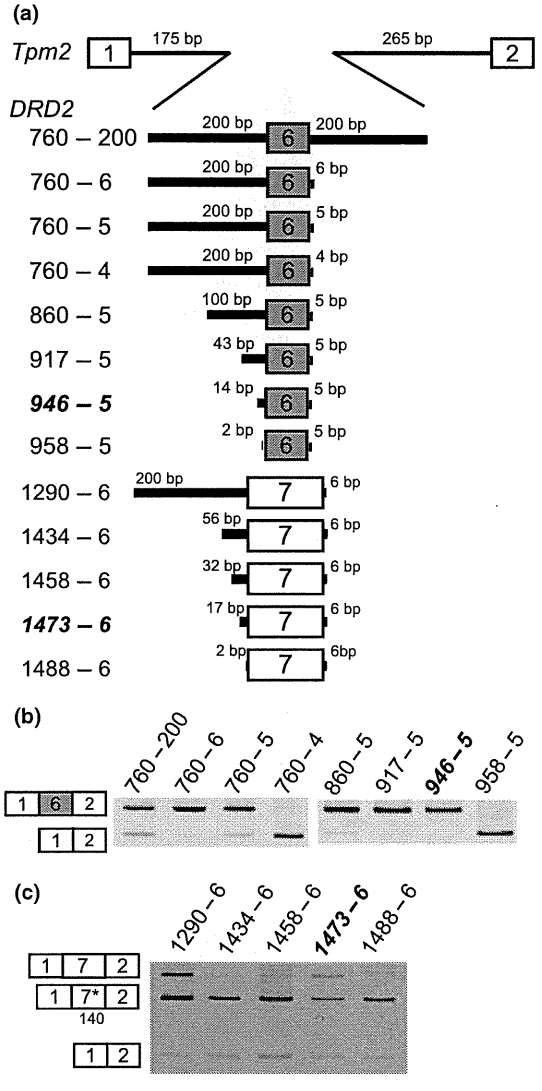


Fig. 3 Identification of DRD2 intronic regions which are necessary for the splicing of exon 6 and exon 7. (a) Structure of the *Tpm2*-based heterologous minigenes. The positions of the inserted nucleotides in introns 5, 6 and 7, as well as the numbers of base pairs in the fragments, are indicated. (b, c) Representative results from identification of splice variants using *Tpm2*-based heterologous minigenes in HEK293 cells. The white box 7* shows a shorter exon 7 lacking the first 140 nucleotides.

Discussion

Previous studies have shown that the functions of two splice variants of DRD2, D2L and D2S, differ in their biochemical properties and physiological functions (Senogles 1994; Guiramand *et al.* 1995; Khan *et al.* 1998; Usiello *et al.* 2000; Wang *et al.* 2000; Centonze *et al.* 2002, 2004; Lindgren *et al.* 2003; Senogles *et al.* 2004; Hranilovic *et al.* 2008); however, it is unclear what regulates the expression ratio of these isoforms. In this study, we identified PTBP1 as a splicing regulatory protein that reduces the expression of the D2S isoform.

Among the eleven proteins that we over-expressed with the *DRD2* minigene in HEK293 cells, only PTBP1

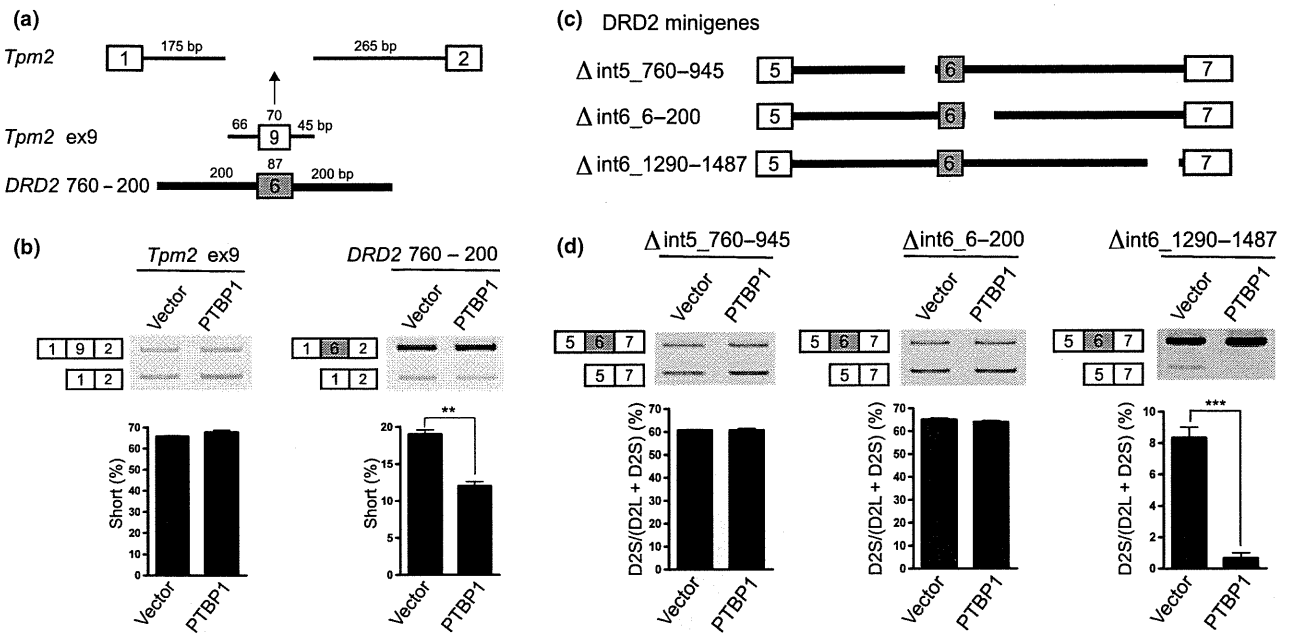


Fig. 4 Splicing regulation by PTBP1 in heterologous minigenes and *DRD2* deletion mutants. (a) Structure of the *Tpm2*-based heterologous minigene. Intronic fragments derived from *DRD2* are indicated by thick lines, whereas those derived from *Tpm2* are indicated by thin lines. (b) Splicing assay results using *Tpm2*-based heterologous minigenes and PTBP1 in HEK293 cells. Bar charts show the quantified percentages of exon exclusion (Mean + SEM, $n = 3$). (c) Structure of the *DRD2* deletion mutants. The positions of the inserted nucleotides in introns 5 and 6 are indicated. (d) Splicing assay results using the *DRD2* deletion mutants and PTBP1 in HEK293 cells. Bar charts show the quantified percentages of D2S (Mean + SEM, $n = 3$). The statistical significance was analyzed using *t*-tests (**$p < 0.01$, ***$p < 0.001$).

produced an altered splicing pattern (Fig. 1b and c). The reduction in the percentage of D2S suggests that PTBP1 enhances the inclusion of the alternative exon 6. Although the effect of PTBP1 was relatively small, this effect was shown to be concentration dependent (Figure S2). We also demonstrated that endogenous PTBP1 regulates DRD2 splicing by knockdown experiments in HEK293 cells with the *DRD2* minigene and in SH-SY5Y cells with the endogenous *DRD2* gene (Fig. 2a and c). Even though the effect of PTBP1 was statistically significant, it was quantitatively small in our splicing assay. Therefore, some other splicing factors may be involved in the splicing regulation of *DRD2*. In addition, the double knockdown of PTBP1 and nPTB suggested that nPTB, a homolog of PTBP1, has little or no effect on the alternative splicing of DRD2 (Fig. 2a). However, because the expression levels of exogenous and endogenous nPTB were remarkably lower than PTBP1 in HEK293 cells, it is still unclear whether nPTB regulates the splicing of DRD2.

Next, we identified the regions responsive to PTBP1, using *Tpm2*-based heterologous minigenes and *DRD2* deletion mutants. Using our heterologous minigenes, the splicing of a *DRD2* fragment containing exon 6 as well as 200 bp-upstream and -downstream intronic regions was altered by PTBP1 (Fig. 4b), similar to the results obtained using the *DRD2* minigene (Fig. 1b and c). In the *DRD2* deletion mutants, PTBP1 had no effect on the splicing of deletion mutants lacking exon 6-flanking regions in intron 5 or 6, whereas PTBP1 still affected the splicing of a deletion mutant lacking the 3′ end of intron 6 (Fig. 4d). These results indicate that exon 6-flanking regions are sufficient for the response to PTBP1, and that both regions in introns 5 and 6 are necessary.

Although PTBP1 is known to bind cytosine and uracil (CU)-rich intronic elements flanking an exon and repress splicing (Wagner and Garcia-Blanco 2001; Sharma *et al.* 2008), in this study PTBP1 appeared to enhance the inclusion of *DRD2* exon 6 rather than repressing the splicing from exon 5 to exon 7. It is noted that intron 5 contains UCUCU (849–853) and intron 6 contains UCUUUCU (32–38) sequences, but we have no evidence that PTBP1 directly binds the pre-mRNA of *DRD2*. Therefore it is possible that PTBP1 may indirectly affect the alternative splicing of *DRD2*.

It was reported that a DRD2 antagonist, haloperidol, increased the expression of D2S (Arnauld *et al.* 1991). The activation of DRD2 is coupled to the inhibition of adenylyl cyclase and cAMP-dependent protein kinase A, and cAMP-dependent protein kinase A has been shown to modulate the nucleocytoplasmic translocation of PTBP1 (Xie *et al.* 2003; Knoch *et al.* 2006). Together with these reports, our results suggest that DRD2 regulates the expression of its isoforms by modulating the localization of PTBP1.

Acknowledgements

This work was supported in part by the Human Frontier Science Program and by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan to S.I. T.S. is supported by a JSPS Research Fellowship for Young Scientists. We thank Dr. Yoshihiro Kino for Tpm2 minigene.

Supporting information

Additional Supporting information may be found in the online version of this article:

Figure S1. The expressions of RNA-binding proteins were confirmed by western blot analysis.

Figure S2. The concentration dependency of the PTBP1 effects.

Table S1. Primer sequences used for cloning.

Table S2. Branch site prediction by ESEfinder 3.0.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

References

Arnauld E., Arsaut J. and Demotes-Mainard J. (1991) Differential plasticity of the dopaminergic D2 receptor mRNA isoforms under haloperidol treatment, as evidenced by in situ hybridization in rat anterior pituitary. *Neurosci. Lett.* **130**, 12–16.

Blasi G., Lo Bianco L., Taurisano P. *et al.* (2009) Functional variation of the dopamine D2 receptor gene is associated with emotional control as well as brain activity and connectivity during emotion processing in humans. *J. Neurosci.* **29**, 14812–14819.

Boutz P., Stoilov P., Li Q. *et al.* (2007) A post-transcriptional regulatory switch in polypyrimidine tract-binding proteins reprograms alternative splicing in developing neurons. *Genes Dev.* **21**, 1636–1652.

Centonze D., Usiello A., Gubellini P., Pisani A., Borrelli E., Bernardi G. and Calabresi P. (2002) Dopamine D2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D2L receptors. *Neuropsychopharmacology* **27**, 723–726.

Centonze D., Gubellini P., Usiello A. *et al.* (2004) Differential contribution of dopamine D2S and D2L receptors in the modulation of glutamate and GABA transmission in the striatum. *Neuroscience* **129**, 157–166.

Cooper T. A. (2005) Use of minigene systems to dissect alternative splicing elements. *Methods* **37**, 331–340.

Grossman J., Meyer M., Wang Y., Mulligan G., Kobayashi R. and Helfman D. (1998) The use of antibodies to the polypyrimidine tract binding protein (PTB) to analyze the protein components that assemble on alternatively spliced pre-mRNAs that use distant branch points. *RNA* **4**, 613–625.

Guiramand J., Montmayeur J., Ceraline J., Bhatia M. and Borrelli E. (1995) Alternative splicing of the dopamine D2 receptor directs specificity of coupling to G-proteins. *J. Biol. Chem.* **270**, 7354–7358.

Guivarc'h D., Vernier P. and Vincent J. (1995) Sex steroid hormones change the differential distribution of the isoforms of the D2 dopamine receptor messenger RNA in the rat brain. *Neuroscience* **69**, 159–166.

Guivarc'h D., Vincent J. and Vernier P. (1998) Alternative splicing of the D2 dopamine receptor messenger ribonucleic acid is modulated by activated sex steroid receptors in the MMQ prolactin cell line. *Endocrinology* **139**, 4213–4221.

Hranilovic D., Bucan M. and Wang Y. (2008) Emotional response in dopamine D2L receptor-deficient mice. *Behav. Brain Res.* **195**, 246–250.

Khan Z., Mrzljak L., Gutierrez A., de la Calle A. and Goldman-Rakic P. (1998) Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proc. Natl Acad. Sci. USA* **95**, 7731–7736.

Kino Y., Washizu C., Oma Y., Onishi H., Nezu Y., Sasagawa N., Nukina N. and Ishiura S. (2009) MBNL and CELF proteins regulate alternative splicing of the skeletal muscle chloride channel CLCN1. *Nucleic Acids Res.* **37**, 6477–6490.

Knoch K., Meisterfeld R., Kersting S., Bergert H., Altkrüger A., Wegbrod C., Jäger M., Saeger H. and Solimena M. (2006) cAMP-dependent phosphorylation of PTB1 promotes the expression of insulin secretory granule proteins in beta cells. *Cell Metab.* **3**, 123–134.

Li Q., Lee J. and Black D. (2007) Neuronal regulation of alternative pre-mRNA splicing. *Nat. Rev. Neurosci.* **8**, 819–831.

Lindgren N., Usiello A., Goiny M., Haycock J., Erbs E., Greengard P., Hokfelt T., Borrelli E. and Fisone G. (2003) Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. *Proc. Natl Acad. Sci. USA* **100**, 4305–4309.

Makeyev E., Zhang J., Carrasco M. and Maniatis T. (2007) The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. *Mol. Cell* **27**, 435–448.

Oomizu S., Boyadjieva N. and Sarkar D. (2003) Ethanol and estradiol modulate alternative splicing of dopamine D2 receptor messenger RNA and abolish the inhibitory action of bromocriptine on prolactin release from the pituitary gland. *Alcohol. Clin. Exp. Res.* **27**, 975–980.

Senogles S. (1994) The D2 dopamine receptor isoforms signal through distinct Gi alpha proteins to inhibit adenylyl cyclase. A study with site-directed mutant Gi alpha proteins. *J. Biol. Chem.* **269**, 23120–23127.

Senogles S., Heimert T., Odife E. and Quasney M. (2004) A region of the third intracellular loop of the short form of the D2 dopamine receptor dictates Gi coupling specificity. *J. Biol. Chem.* **279**, 1601–1606.

Sharma S., Kohlstaedt L., Damianov A., Rio D. and Black D. (2008) Polypyrimidine tract binding protein controls the transition from exon definition to an intron defined spliceosome. *Nat. Struct. Mol. Biol.* **15**, 183–191.

Usiello A., Baik J., Rougé-Pont F., Picetti R., Dierich A., LeMeur M., Piazza P. and Borrelli E. (2000) Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* **408**, 199–203.

Wagner E. and Garcia-Blanco M. (2001) Polypyrimidine tract binding protein antagonizes exon definition. *Mol. Cell. Biol.* **21**, 3281–3288.

Wang Y., Xu R., Sasaoka T., Tonegawa S., Kung M. and Sankoorikal E. (2000) Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. *J. Neurosci.* **20**, 8305–8314.

Xie J., Lee J., Kress T., Mowry K. and Black D. (2003) Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. *Proc. Natl Acad. Sci. USA* **100**, 8776–8781.

Zhang Y., Bertolino A., Fazio L. *et al.* (2007) Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proc. Natl Acad. Sci. USA* **104**, 20552–20557.

TOPICS トピックス

記憶力を良くしたい！
——インスリン様成長因子II (IGF-II) のはたらき

*Nature*誌2011年1月27日号掲載記事を読む

Chen, D. Y., Stern, S. A., Garcia-Osta, A., Saunier-Rebori, B., Pollonini, G., Bambah-Mukku, D., Blitzer, R. D., Alberini, C. M. A critical role for IGF-II in memory consolidation and enhancement. *Nature*, **469**, 491–497 (2011).

記憶力を高めるくすりがあると言われれば，そのような物質を欲しいと思う人がほとんどではないだろうか？私が受験勉強をする学生だったころには，集中力を高める物質として，カフェインに効果があると聞いてコーヒーを飲んでいたことを思い出す。最近では，さまざまな精神治療薬に記憶力を高める効果があるのでは？　と考え，臨床でも役立つような記憶薬の開発に取り組んでいる人たちもいる。これから紹介する*Nature*誌の1月27日号では，Dilon Y. Chenらが，インスリン様成長因子II (IGF-II) というポリペプチドに記憶力を高める働きがあることを報告している。

抑制性忌避学習実験とは？

この論文では，ラットを使った学習実験で，記憶のメカニズムについて研究している。図1に示したように，ラットを入れる実験用の箱の中には，安全な場所（壁は白色で明るい）と床から軽微な電気ショックを与える場所（壁は黒色で暗い）を作り，仕切りで区切っておく。実験では，はじめにラットを安全な場所に置き，仕切りを開ける。そうすると，何も知らない哀れなラットは10～20秒で暗い電気ショック領域に入り，電気ショック（0.6 mAで2秒

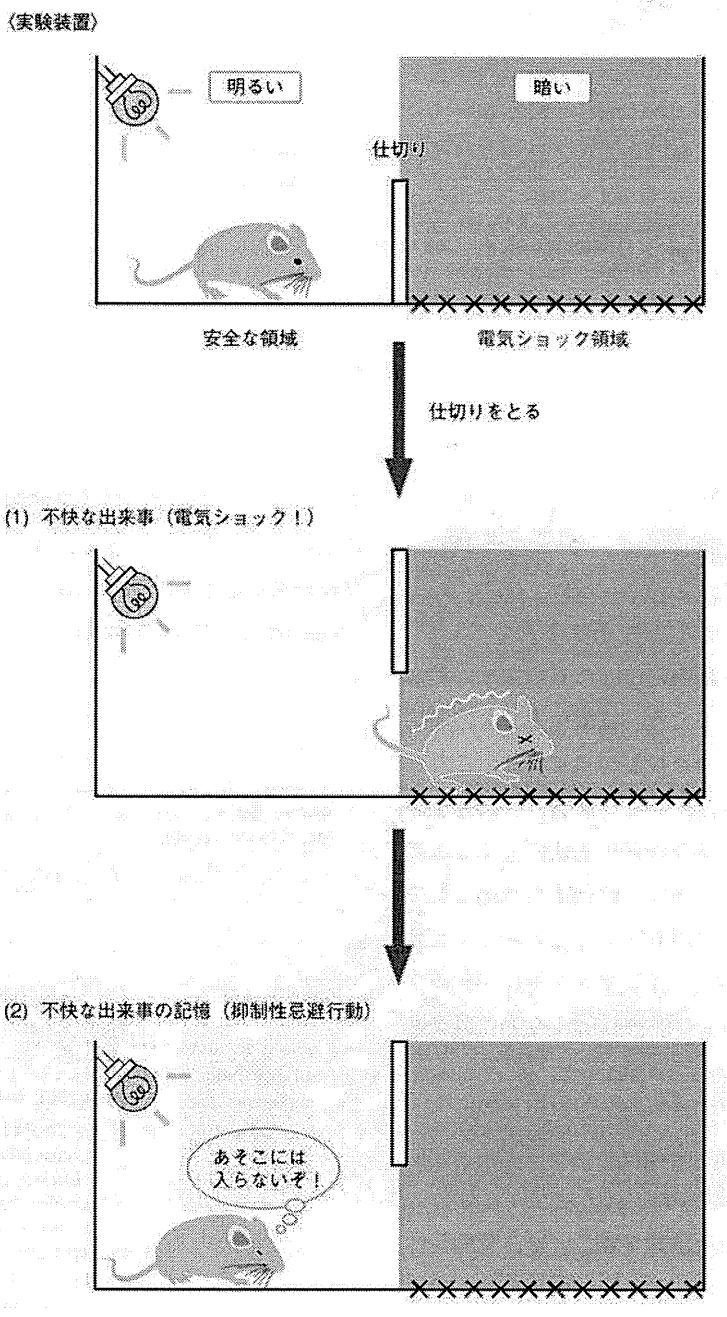


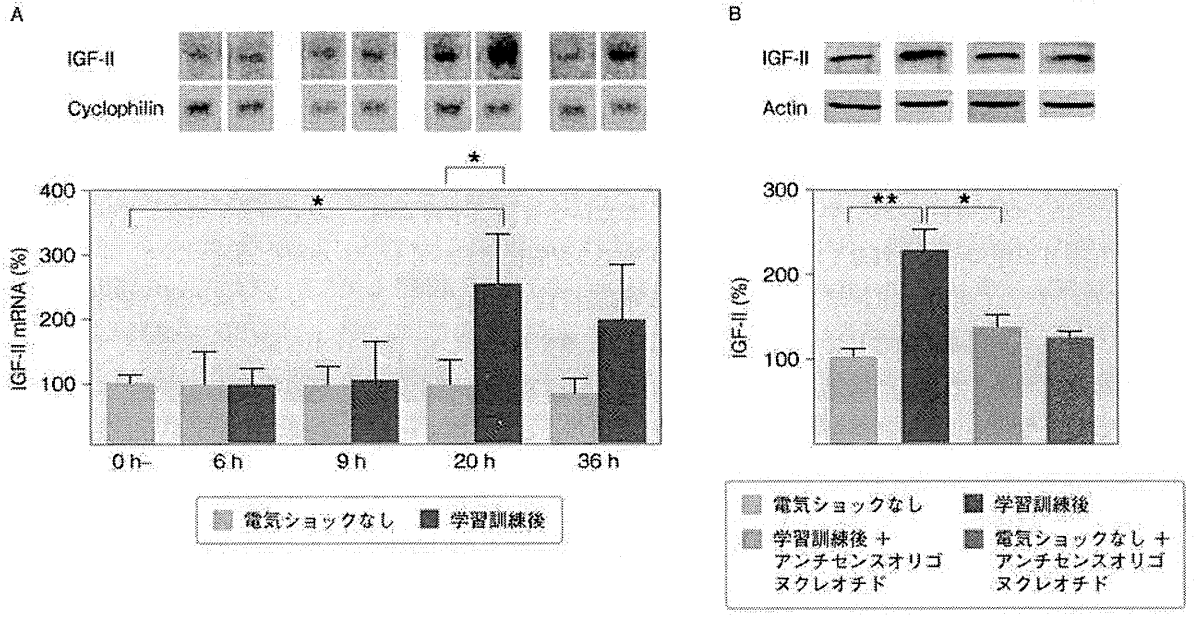
図1　抑制性忌避学習実験

間）を受けてしまう。ラットは，習性として暗い所を好むのである。しかし，この不快な“学習訓練”を受けた後では，ラットは危険な領域を認識して避けるようになる。つまり，電気ショックを受けたことを「記憶」しているかについて，実験装置に入れたときに，何秒後に電気ショック領域に入るかを計測して判定するというのが，この学習実験（「抑制性忌避学習」という）の概略だ。“訓練”の1日後に実験すると，ラットは約5分間，電気ショックを受けた場所に入ろうとしない。しかし，日が経つにつれて，段々電気ショックのことを忘れてしまい，3～4週間で元の状態に戻って，また電気ショック領域に入ってしまう。

記憶にはタンパク質合成が必要である

さて，ノーベル賞学者E. Kandelは，アメフラシ（軟体動物）を使った神経回路の研究から，“記憶”には遺伝子の転写誘導とタンパク質の合成が必要であることを明らかにした。神経細胞が活性化されると，神経細胞間の情報伝達効率が変化する。その変化が固定化されて長期にわたって継続する“記憶”になるためには，新たに作り出されたタンパク質がシナプスの変化を引き起こすことが必要なのである〔活性化された神経細胞では，必要な遺伝子（DNA）が転写因子に選び出されて，メッセンジャーRNAへと転写され，タンパク質が合成される〕。そこで重要な役割を果たすのは，必要な遺伝子を選び出す転写因子である。記憶に関連する転写因子としては，これまでに，cAMP応答因子結合タンパク質（CREB）とCCAATエンハンサー結合タンパク質（C/EBP）が知られている。論文では，このうち，C/EBPについて調べられている。C/EBPは記憶の固定化に働くことが知られていたが，転写誘導する遺伝子については，実際に何が必要なのかはよくわかっていなかった。

この研究では，インスリン様成長因子II（IGF-II）が重要であることが明らかになる。その名前のとおり，これまでは成長因子としての役割に光が当てられてきたが，このIGF-IIは脳内に多く存在する分子で，加齢とともに量が減っていくことが知られている。また，ニジマスのIGF-II遺伝子についての解析（1998年）から，遺伝子の内部（プロ



A: 学習実験後のラットの脳内では，20時間後をピークにIGF-II mRNAの量が増加していた（ノーザンブロット解析）

B: タンパク質の量を見ても（ウェスタンブロット解析），学習実験をしたラットの脳ではIGF-IIが増えていた。しかし，C/EBPのアンチセンスオリゴヌクレオチドを脳に投与したラットでは，この増加が見られなかった

Cyclophilinとactinは量に変化がない対照として示されている

（★印は統計的に有意な差であることを示している（*P値<0.05，**P値<0.01））

（Chenらの図より改変）

図2　学習実験後のラット脳内におけるC/EBPのIGF-II転写誘導

モーター領域）にC/EBPが結合するDNA配列があることがわかっており，記憶に関連する新たな遺伝子として著者たちは興味をもった。

学習によってラットの脳内で起こる変化

著者たちは，学習訓練を行った後のラットの脳を解剖して，海馬（脳の記憶装置）での遺伝子発現を解析した。その結果，転写因子C/EBPの量が増加することを発見した。C/EBPの量は，訓練の後，約20時間をピークに増加して48時間後には元に戻る。ここで，彼らは，IGF-IIについても調べたところ，C/EBPと同じように，学習実験の20時間後をピークにして増加が見られた（図2A）。では，IGF-IIはC/EBPによって発現誘導されるのか？　IGF-IIとC/EBPの関係を証明するために，彼らは海馬のC/EBPをなくす実験を行った。アンチセンスオリゴヌクレオチドという物質を海馬に注入してC/EBP遺伝子の発現をブロックしたところ，海馬のC/EBPがなくなっただけではなく，学習訓練後のIGF-IIの増加が見られなくなった（図2B）。また，C/EBPはIGF-II遺伝子のプロモーター領域に結合していることも確認された。これらの結果から，学習訓練を受けたラットの海馬ではC/EBPの量が増加し，C/EBPがIGF-II遺伝子を転写誘導することが明らかになった。

IGF-IIは記憶力を高める

学習訓練の後に量が増えていることがわかったIGF-IIは，記憶に必要な分子なのだろうか？　この疑問について，今度は，海馬内でIGF-IIをなくす実験とIGF-IIの量を増やす実験を行っている。

まず，先ほども出てきたアンチセンスオリゴヌクレオチドを使って海馬内のIGF-IIをなくす実験を行った（図3A）。その結果，学習訓練後24時間までにIGF-IIの発現をブロックすると，電気ショックを記憶できなくなった。IGF-IIをブロックするタイミングは重要で，実験後4日経った後だと，記憶を抑える効果はなくなる。つまり，IGF-IIは，ラットが“不快な出来事”を記憶する際，4日後までに脳内で起こる変化に重要な働きをするということがわかる。

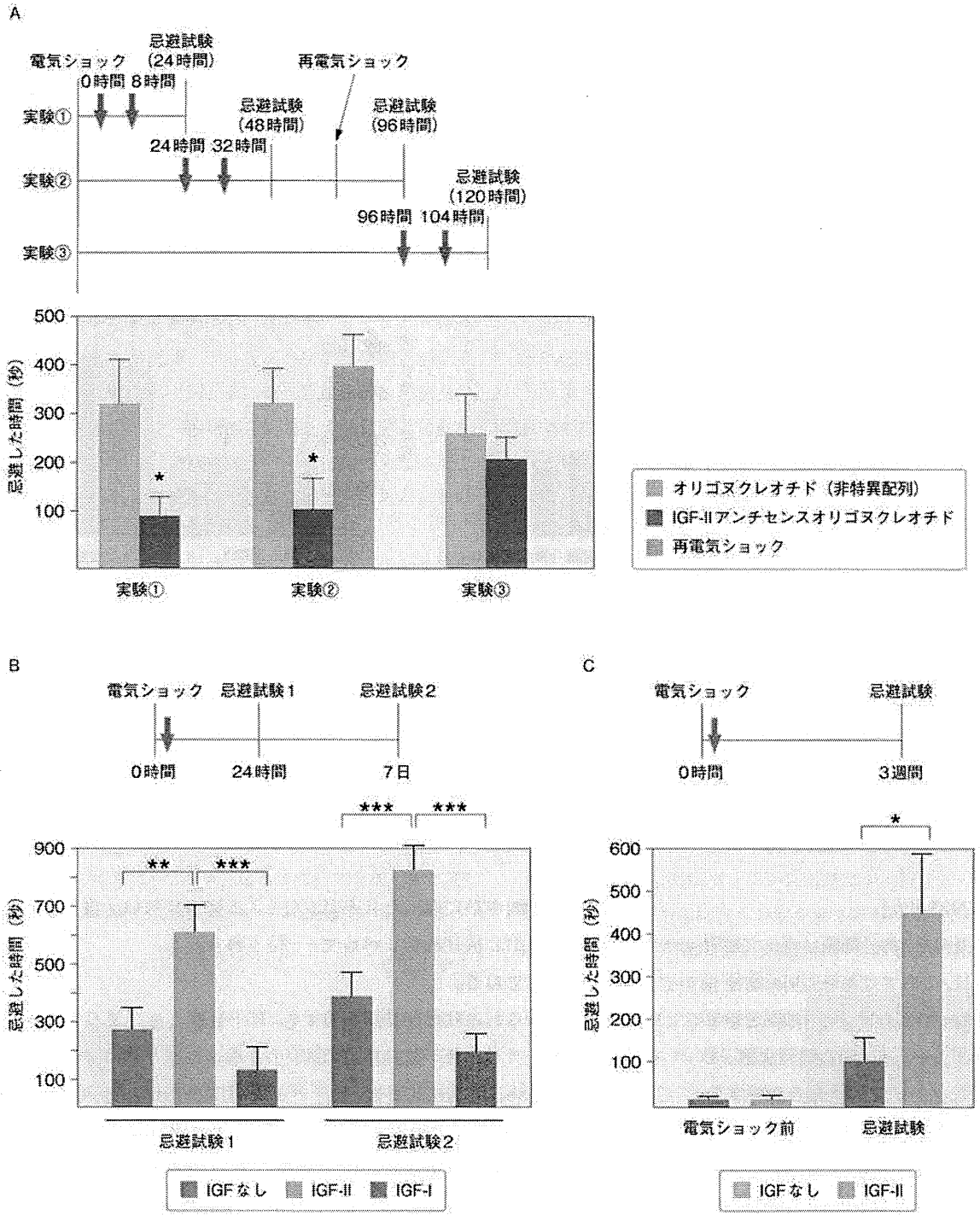
また，驚くべきことに，IGF-II（タンパク質）を海馬内に注入すると，記憶が改善することも明らかになった（図3B，図3C）。IGF-IIは細胞の外に分泌されて，他の細胞に作用するポリペプチドである。脳内に注入されて神経細胞に影響を与えると考えられる。学習実験の直後にIGF-IIを注入（両方の海馬に25 ngずつ）すると，ラットが電気ショックを避ける行動が強まり（1日後の回避行動は通常5分程度のものが15分程度に上昇），なおかつ普通は学習実験の効果がなくなる3週間後においても，ラットは高い回避行動を“記憶”していた。IGF-IIは“記憶を強化”して，“物忘れ”を防いだと言える。数週間もその効果が続くということは，驚くべきことである。

IGF-IIの脳内投与が記憶への効果を示すのは，学習訓練の直後に投与したときに限られていた。しかし，訓練が終わってしばらく経ってからでも効果が見られた条件があった。学習訓練の1日後にラットを実験装置に入れて（このときには電気ショックは与えない），その直後にIGF-IIを注入すると，その後の忌避行動が著しく改善したのである。実際の不快な出来事ではなくて，その記憶を“想起”した（思い出した）とき（「記憶の再固定化」と言う）でも，投与されたIGF-IIによって記憶の保持が強化されたのである（図4）。記憶の再固定化は，人間も日常的に行っている。昔の記憶が消えていくのを防ぐことができるとしたら，非常に魅力的なことであろう。

IGF-IIの作用機構

さて，IGF-IIは脳内の何に作用して，記憶力を高めているのだろうか？　そのメカニズムについても，論文では報告している。IGF-IIは細胞の外に分泌されて他の細胞に作用するが，IGF-IIの作用には，神経細胞表面でIGF-IIと結合するIGF-II受容体が必要だった。IGF-IIが受容体に結合すると，情報伝達経路を介して細胞内でさまざまな変化が起こる。そのうちで記憶の固定に重要なのは，シナプスの構造変化ではないかと考えられる。シナプスは神経細胞同士が結合して，情報を交換する場所になっている。

シナプスの構造変化への作用を示唆する第一の証拠として，IGF-IIの記憶への作用にはシナプスでのタンパク質合成に関与するArc（細胞骨格関連タンパク質）が必要であった。第二に，海馬のシナプスを分離して解析したところ，IGF-IIを投与して“記憶力の上がったラット”のシナプスでは，GSK3（グリコーゲンシンターゼキナーゼ）という調節タンパク質の活性が大幅に活性化し，AMPA（「α-アミノ-3-ヒドロキシ-5-メソオキソザール-4-プロピオン酸」の略）受容体の量が増加している



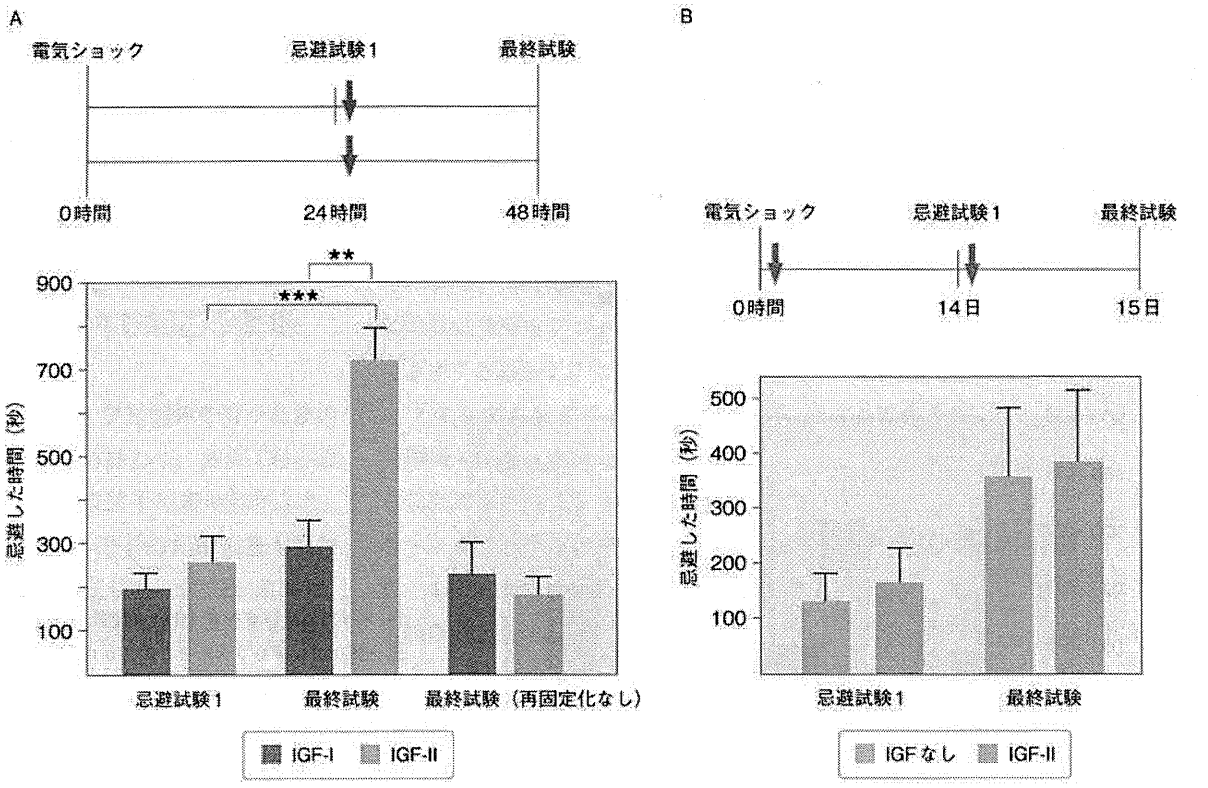
A: IGF-IIを脳内からなくすと，学習効果が見られなくなった。3つの実験（①〜③）において，↓で示したタイミングでIGF-IIアンチセンスオリゴヌクレオチドを海馬に投与して学習効果による忘避時間の増減を測ったところ，実験①と実験③では電気ショックを忘れて忘避時間が短くなった。IGF-IIアンチセンスオリゴヌクレオチドを96時間経った後に加えた実験③では，効果が見られなかった。また，再度電気ショックを与えると学習して忘避行動の時間が長くなるため（実験②の結果），アンチセンスオリゴヌクレオチドの効果は可逆的なものであることがわかる

B, C: IGF-IIの脳内での量を増やすと，記憶が強化された。電気ショックを与えた直後（↓）のラットの海馬にIGF-IIを投与すると忘避行動の時間が長くなり（通常の倍），またラットがほとんど実験のことを忘れてしまう3週間後にもIGF-II投与群は忘避行動を忘れずにいた。対照として投与したIGF-Iでは効果が見られなかった

〔★印は統計的に有意な差であることを示している（* P値<0.05, ** P値<0.01, *** P値<0.001）〕

（Chenらの図より改変）

図3　学習実験を使ったIGF-IIの効果の実証



A: 上に図示したスケジュールで実験を行った。忌避行動を試験した直後のラットの脳（海馬）にIGF-IIを投与したところ（↓），忌避行動が大きく改善された。忌避行動試験では電気ショックは与えていないことに注意してほしい。ラットが記憶を"再固定化"した直後のIGF-IIに効果があったということである。再固定化なしに，24時間後にIGF-IIを投与するだけでは，効果は見られない

B: ラットの脳内で記憶を再固定化できる時間には限りがあるようで，Aと同様の実験を14日後に行った場合では，IGF-IIの効果は見られなかった

（Chenらの図より改変）

図4　IGF-IIの記憶の再固定化に対する効果

ことが観察された。

記憶の基盤を，神経細胞レベルで解明する試みは，これまで海馬の切片を使った実験で行われてきた。神経細胞を電気的に刺激すると，細胞間の情報交換，すなわちシナプス伝達の効率を測定することができる。神経細胞を高頻度で刺激して活性化すると，シナプスの伝達効率が増強される。これをLTP（長期増強）という。先に述べたAMPA受容体は，グルタミン酸と結合する受容体型イオンチャネルで，LTPで重要な役割を果たすチャネルである。では，海馬の切片にIGF-IIを添加するとどうなるか？著者たちの解析の結果，IGF-IIを添加した海馬の切片では，LTPが促進された。動物実験で明らかになったIGF-IIの記憶への作用が，神経細胞レベルでも確認されたのである。

現時点で考えられるIGFが作用するメカニズムとしては，IGF-IIの添加によってArcやGSK3が活性化され，シナプスでのAMPA受容体の量が増加し，シナプス伝達の効率を向上させ，記憶力を高めたと考えられる。

IGF-II（インスリン様成長因子II）については，その名前にもあるように，細胞の成長因子としての機能に焦点が当てられてきた。今回のIGF-IIの効果についても，IGF-IIによって新しい神経細胞が成長してできた可能性も完全には否定できない。記憶力を高めるメカニズムについては，さらなる研究が期待される。

IGF-IIは，インスリンに似た比較的小さなポリペプチドである。IGF-IIやIGF-II受容体は，今後の研究次第では，認知機能増強治療，記憶薬開発のための新しいターゲットになるかもしれない。糖尿病の患者にインスリンを注射するように，認知機能が低下した患者にIGF-IIを注射して記憶力治療ができるとしたら，こんなにすばらしいことはない。

二井 勇人 *Eugene Futai*
東北大学 大学院農学研究科 准教授