were cut on a freezing microtome, mounted on glass slides from a 0.2% gelatin solution, and counterstained with cresyl violet. Sections were photographed and prints were digitized (Fig. 1B).
12. The changes in optical signal likely represent predominantly the electrical activity of neurons (synaptic potentials and spikes), because they coincide with field potentials recorded over the same area of the slice (Fig. 1C). The spatial resolution of the measurement is not sufficient to detect the behavior of individual neurons. Therefore, no inferences can be made concerning the neuronal properties of the underlying entorhinal network.
13. In this slice, inhibition was partly suppressed by 5 μM bicuculline. Comparable observations were obtained in 23 slices (out of 28 of horizontal slices obtained from the ventral part of the brain, 2.4 mm to 3.0 mm dorsal to the interaural plane) under similar conditions and in 26 slices (out of 31) with only 1 μM of bicuculline. Without bicuculline (n = 56), the number of reverberating cycles was always limited to one, generally without subsequent activation of the hippocampus. Bicuculline could have altered the stability of the preparation and resulted in epileptiform activity. However, we concluded that this was not the case because (i) no activity was recorded before stimulation to EC was applied; (ii) stimulation of EC evoked activity only for 600 ms, and no activity was measured afterward; (iii) no significant change in propagation patterns was seen during sessions (up to 2 hours); (iv) directly adjacent slices under similar conditions consistently did not show any oscillations; and (v) no overall oscillatory activity is seen in the hippocampus of the same slice [E. W. Lothman, E. H. Bertram III, J. L. Stringer, *Prog. Neurobiol.* **37**, 1 (1991); U. Heinemann *et al.*, *Epilepsy Res. Suppl.* **7**, 273 (1992).
14. The movie is available at http://science-mag.aaas.org/science/feature/beyond/#iijima.
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17. Even in the presence of bicuculline (1 to 5 μM), membrane depolarizations were generally followed by long-lasting hyperpolarizations, which implies that the inhibitory system is not fully suppressed. In normal solution, stimulus frequencies of 1 Hz and higher can overcome the local inhibition (*16*). Thus, with a partial suppression of inhibition, the reverberating circuit at 0.1 Hz might be sufficient to overcome inhibition, resulting in activation of the perforant pathway.
18. In similar preparations, stimulation of either CA3 or the subiculum led to activation of the deep layers of EC and subsequent reverberating activity. Also, direct stimulation laterally in the deep layers of LEA resulted in the spread of activity in the deep layers to MEA, subsequent spread into superficial layers of MEA, followed by activation of the reverberating circuit.
19. We thank D. G. Amaral, A. H. M. Lohman, F. H. Lopes da Silva, R. Malinow, and W. J. Wadman for their suggestions and discussion. M.P.W. was supported by a travel grant from the Agency of Industrial Science and Technology (AIST) of Japan and the Netherlands Organization for Scientific Research (NWO).

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TAB1: An Activator of the TAK1 MAPKKK in TGF-β Signal Transduction

Hiroshi Shibuya, Kyoko Yamaguchi, Kyoko Shirakabe, Akane Tonegawa, Yukiko Gotoh, Naoto Ueno, Kenji Irie, Eisuke Nishida, Kunihiro Matsumoto*

Transforming growth factor–β (TGF-β) regulates many aspects of cellular function. A member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, TAK1, was previously identified as a mediator in the signaling pathway of TGF-β superfamily members. The yeast two-hybrid system has now revealed two human proteins, termed TAB1 and TAB2 (for TAK1 binding protein), that interact with TAK1. TAB1 and TAK1 were co-immunoprecipitated from mammalian cells. Overproduction of TAB1 enhanced activity of the plasminogen activator inhibitor 1 gene promoter, which is regulated by TGF-β, and increased the kinase activity of TAK1. TAB1 may function as an activator of the TAK1 MAPKKK in TGF-β signal transduction.

The mitogen-activated protein kinase (MAPK) pathway is a conserved eukaryotic signaling module that converts receptor signals into various outputs. This pathway includes three protein kinases: MAPKKK, MAPKK, and MAPK; MAPK is activated through phosphorylation by MAPKK, which is first activated by MAPKKK (*1*). A member of the MAPKKK family, TAK1 (TGF-β–activated kinase 1), that functions in the signaling pathway of TGF-β superfamily members has been identified (*2*). TGF-β signals through a heteromeric complex of type I and type II TGF-β receptors, which are transmembrane proteins that contain cytoplasmic serine- and threonine-specific kinase domains (*3*). However, little is known at the molecular level of the signaling mechanism downstream of the TGF-β receptors.

To analyze the TAK1-dependent pathway that functions in TGF-β signal transduction, we used the yeast two-hybrid system (*4*) to search for proteins that directly interact with TAK1. Yeast cells were co-transformed with a TAK1 expression vector, encoding the LexA DNA binding domain fused to TAK1 (*5*), and plasmids containing human brain cDNA expression library clones fused to DNA encoding the GAL4 activation domain (GAD). Twenty-six positive clones encoding two distinct proteins, named TAB1 and TAB2, were obtained from ~1×10^6 transformants. The GAD fusion proteins expressed by the two classes of library isolates will be referred to as GAD-TAB1 and GAD-TAB2. To localize the regions in TAK1 responsible for interaction with TAB1 and TAB2, we examined a set of LexA-TAK1 deletion chimeras in two-hybrid assays (Fig. 1A). These studies revealed that TAB1 and TAB2 interact with the NH_2- and COOH-terminal domains of TAK1, respectively.

Proteins that interact with TAK1 may include both upstream regulators and downstream targets. If either TAB1 or TAB2 has a role in the activation of TAK1, one would expect that co-expression with TAK1 would influence the activity of the latter in yeast. We developed an in vivo system for assaying mammalian MAPKKK activity in the yeast pheromone-induced MAPK pathway (*2*, *6*). An activated form of TAK1 (TAK1ΔN) is able to substitute for Ste11p MAPKKK activity (*7*). Expression of full-length TAK1, however, fails to rescue the *ste11*Δ mutation, suggesting that yeast cells do not possess an activator of TAK1 (*2*). With the use of the yeast MAPK pathway, both GAD-TAB1 and GAD-TAB2 constructs were tested for their ability to complement the *ste11*Δ mutation in the presence of TAK1. Co-expression of GAD-TAB1 with TAK1 rescued the Ste11p deficiency, whereas GAD-TAB2 had no effect (Fig. 1B). These results indicate that TAB1 augments the activity of TAK1.

To determine whether TAB1 increases TAK1 activity in yeast cells, we transformed cells expressing or not expressing

H. Shibuya, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, and Precursory Research for Embryonic Science and Technology, Research Development Corporation of Japan, Hikari-dai, Kyoto 619-02, Japan.
K. Yamaguchi, K. Irie, K. Matsumoto, Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan.
K. Shirakabe, Y. Gotoh, E. Nishida, Department of Genetics and Molecular Biology, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.
A. Tonegawa and N. Ueno, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan.

*To whom correspondence should be addressed. E-mail: g44177a@nucc.cc.nagoya-u.ac.jp

TAB1 with expression constructs of TAK1 or a catalytically inactive TAK1 mutant [TAK1(K63W), in which Lys[63] in the adenosine triphosphate–binding site is replaced by Trp (*2*)], each carrying a hemagglutinin (HA)–derived COOH-terminal epitope (*8*). Kinase assays with the immunoprecipitated TAK1-HA and TAK1(K63W)-HA revealed that TAB1 increased the kinase activity of TAK1 (Fig. 1C). This increased activity was not observed in immune complexes from cells expressing TAK1(K63W) and TAB1, indicating that the observed kinase activity is intrinsic to TAK1, These data suggest that TAB1 activates the kinase activity of TAK1 by directly binding to its catalytic domain.

To obtain the full-length coding sequence of TAB1, we screened a human kidney cDNA library with the partial cDNA insert obtained from the yeast two-hybrid system. Two independent clones yielded a 3.1-kb cDNA that contained an open reading frame beginning with an initiator methionine codon that conformed to the Kozak consensus sequence (*9*). This open reading frame predicted a protein of 504 amino acids with a molecular size of 55 kD (Fig. 2A) that showed no marked sequence similarity to known proteins and contained no motifs that might indicate a biochemical function. We analyzed the distribution of TAB1 mRNA in various human tissues by Northern blot analysis. A major transcript of ~3.5 kb was detected in all tissues examined (Fig. 2B).

To confirm that association of TAB1 and TAK1 occurs in mammalian cells, we transiently transfected MC3T3-E1 murine osteoblastic cells (*10*) with expression plasmids encoding TAK1 tagged at the NH_2-terminus with the HA epitope (HA-TAK1) (*2*) and MYC epitope–tagged TAB1 (MYC-TAB1) (*11*). Cell extracts were subjected to immunoprecipitation with antibodies to HA or MYC, and each immunoprecipitate was probed with antibodies to MYC. Similar amounts of MYC-TAB1 were detected in each immunoprecipitate (Fig. 3A), revealing that TAB1 can be co-immunoprecipitated with TAK1. Reciprocal experiments in which immunoprecipitated proteins were probed with antibodies to HA confirmed the association of TAB1 with TAK1 (Fig. 3A). Thus, TAB1 associates with TAK1 in mammalian cells as well as in yeast.

We investigated whether TAB1 overexpression increases the kinase activity of TAK1 in mammalian cells. MC3T3-E1 cells were transiently transfected with the HA-TAK1 vector either with or without the MYC-TAB1 vector. The HA-TAK1 protein was immunoprecipitated from transfected cells that had been incubated in the absence or presence of TGF-β. Portions of the immunoprecipitates were subjected to immunoblot analysis with antibodies to HA to confirm that equivalent amounts of HA-TAK1 were present in all immune complexes (Fig. 3B). Co-expression of TAB1 with TAK1 had no detectable effect on the amount of TAK1. In vitro kinase assays of the TAK1 immunoprecipitates revealed that TAK1 activity was increased in cells co-transfected with the TAB1 vector even in the absence of TGF-β (Fig. 3B). The extent of activation of TAK1 by TAB1 overexpression was similar to that observed in TGF-β–stimulated cells expressing HA-

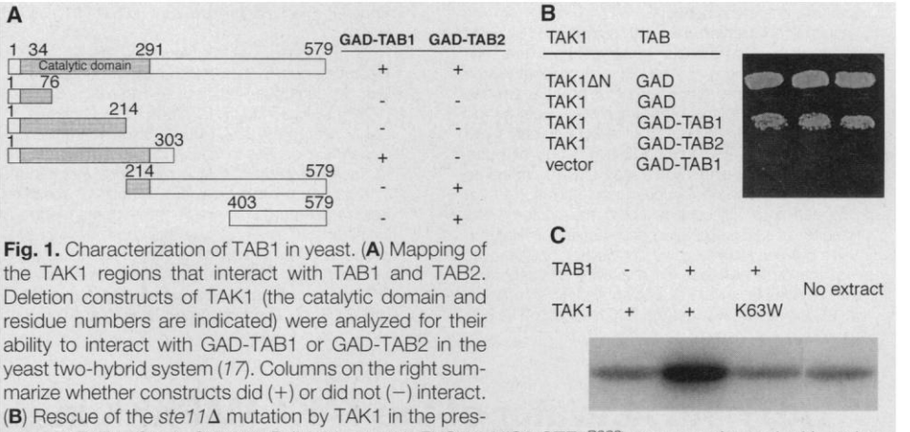

Fig. 1. Characterization of TAB1 in yeast. (**A**) Mapping of the TAK1 regions that interact with TAB1 and TAB2. Deletion constructs of TAK1 (the catalytic domain and residue numbers are indicated) were analyzed for their ability to interact with GAD-TAB1 or GAD-TAB2 in the yeast two-hybrid system (*17*). Columns on the right summarize whether constructs did (+) or did not (−) interact. (**B**) Rescue of the *ste11Δ* mutation by TAK1 in the presence of TAB1. Strain SY1984-P (*his3Δ ste11Δ FUS1p::HIS3 STE7*^{P368}) was transformed with various plasmids (*18*), and the transformants were streaked onto SC−His plates and incubated at 30°C. The columns to the left indicate whether the transformants expressed TAK1ΔN or TAK1, and whether GAD-TAB1 or GAD-TAB2 was also expressed. The panel to the right shows the growth of cells on SC−His plates. Each patch represents an independent transformant. Plasmids were as follows: row 1, pNV11-HU11 (TAK1ΔN) and pGAD10 (GAD) (Clontech); row 2, pNV11-HU11F (TAK1) and pGAD10; row 3, pNV11-HU11F and pGAD-TAB1; row 4, pNV11-HU11F and pGAD-TAB2; and row 5, pNV11 (vector) and pGAD-TAB1. (**C**) Activation of TAK1 by TAB1 in yeast. Yeast strain SY1984 was transformed with various plasmids as indicated (*8*). Transformants received the empty vector (YEpGAP112) (−) or pGAP-HTH9M (+) (*8*) encoding TAB1. TAK1-HA (+) or TAK1(K63W)-HA (K63W) was immunoprecipitated from each cell extract, and immunoprecipitates were subjected to in vitro kinase assays (*19*). The activity of immunoprecipitated TAK1 was assayed by its ability to activate recombinant XMEK2 (SEK1), which, in turn, was assayed by its ability to phosphorylate recombinant catalytically inactive (KN)–p38 (MPK2) (*2*). After electrophoresis, the phosphorylation of KN–p38 (MPK2) was detected by autoradiography. "No extract" indicates the kinase assay performed without yeast extract and corresponds to the basal activity of XMEK2. Experiments were repeated at least three times with similar results.

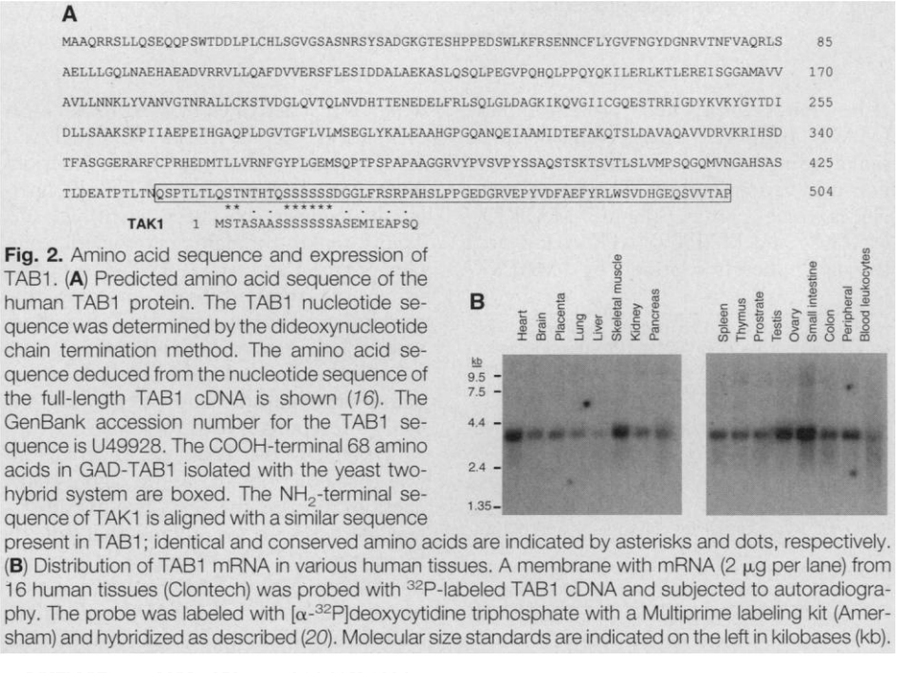

Fig. 2. Amino acid sequence and expression of TAB1. (**A**) Predicted amino acid sequence of the human TAB1 protein. The TAB1 nucleotide sequence was determined by the dideoxynucleotide chain termination method. The amino acid sequence deduced from the nucleotide sequence of the full-length TAB1 cDNA is shown (*16*). The GenBank accession number for the TAB1 sequence is U49928. The COOH-terminal 68 amino acids in GAD-TAB1 isolated with the yeast two-hybrid system are boxed. The NH_2-terminal sequence of TAK1 is aligned with a similar sequence present in TAB1; identical and conserved amino acids are indicated by asterisks and dots, respectively. (**B**) Distribution of TAB1 mRNA in various human tissues. A membrane with mRNA (2 μg per lane) from 16 human tissues (Clontech) was probed with ^{32}P-labeled TAB1 cDNA and subjected to autoradiography. The probe was labeled with [α-^{32}P]deoxycytidine triphosphate with a Multiprime labeling kit (Amersham) and hybridized as described (*20*). Molecular size standards are indicated on the left in kilobases (kb).

TAK1 alone. Together, these observations indicate that TAB1 may be a positive regulator of TAK1.

TAK1 participates in the regulation of transcription by TGF-β (*2*). TGF-β rapidly increases the amount of plasminogen activator inhibitor 1 (PAI1) mRNA (*12*). Overexpression of an activated form of TAK1 (TAK1ΔN) constitutively activates a reporter construct containing the luciferase gene under the control of the TGF-β–inducible PAI1 gene promoter (*2*). We examined whether overexpression of TAB1 also resulted in activation of the luciferase reporter gene. Mv1Lu mink lung epithelial cells were transfected with the luciferase construct (p800neoLUC) (*13*) as well as with expression plasmids encoding TAB1 (*14*) or TAK1 (*2*). Overexpression of TAB1 together with TAK1 induced the expression of the reporter gene even in the absence of TGF-β, whereas overexpression of TAK1 or TAB1 alone had little effect on constitutive luciferase activity (Fig. 4A). These results indicate that TAB1 enhances the activity of TAK1 in mammalian cells. Overexpression of the TAK1(K63W) mutant inhibited the TGF-β–induced increase in luciferase activity (*2*), presumably by sequestering essential elements in the pathway. Overexpression of TAB1 abolished the inhibitory effect of TAK1(K63W) (Fig. 4A), suggesting that TAB1 may be sequestered by overexpression of TAK1(K63W).

The COOH-terminal 68 amino acids of TAB1 [TAB1(437–504)] were sufficient for binding to and activating TAK1, suggesting that the NH_2-terminal domain of TAB1 may play a regulatory role in TAB1 function. To test this hypothesis, we generated a truncated version of TAB1 lacking the COOH-terminal TAK1-binding domain [TAB1(1–418)]. TAB1(1–418) did not associate with or activate TAK1 in the yeast two-hybrid and MAPKKK activation assays (*15*). Overexpression of TAB1(1–418) in Mv1Lu cells suppressed the reporter gene activity induced by TGF-β stimulation (Fig. 4B). Thus, TAB1(1–418) acts as a dominant-negative inhibitor of TGF-β–induced gene expression. These results suggest that TAB1 participates in TGF-β signaling.

We have not succeeded in activating TAK1 (immunoprecipitated from yeast cells overexpressing TAK1-HA) by bacterially synthesized TAB1 protein in a cell-free system. Although the mechanism by which TAB1 activates TAK1 remains to be elucidated, one possibility is that TAB1 binding to TAK1 induces an activating conformational change. Deletion of 20 amino acids from the NH_2 terminus of TAK1 results in constitutive activation of the protein kinase, suggesting that the NH_2-terminal domain may engage the catalytic domain to inhibit kinase activity (*2*). TAB1 may dislodge this negative regulatory domain of TAK1 from the catalytic domain. The COOH terminus of TAB1, which acts as the TAK1 binding site, contains a region rich in serine and threonine residues similar to that present in the NH_2-terminal domain of TAK1 (Fig. 2A). Thus, TAB1 may be an important signaling intermediate between TGF-β receptors and the TAK1 MAPKKK.

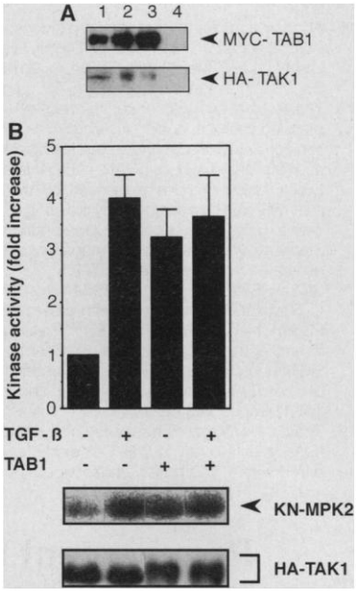

Fig. 3. Interaction of TAB1 with TAK1 in mammalian cells. (**A**) In vivo association of TAB1 with TAK1. MC3T3-E1 cells were transiently transfected with mammalian expression vectors encoding HA-TAK1 (*2*) and MYC-TAB1 (*11*). Cell extracts were subjected to immunoprecipitation with monoclonal antibody 12CA5 to HA (lane 2), monoclonal antibody 9E10 to MYC (lane 3), or control nonimmune immunoglobulin G (lane 4). The immune complexes were washed, resolved by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose, and subjected to immunoblot analysis with either antibodies to MYC (upper panel) or HA (lower panel). The cell extract was also directly subjected to immunoblot analysis (lane 1). (**B**) Activation of the kinase activity of TAK1 by co-expression with TAB1. MC3T3-E1 cells were transiently transfected with the HA-TAK1 vector in the presence (+) or absence (−) of the MYC-TAB1 vector. The cells were incubated with (+) or without (−) TGF-β1 (20 ng/ml) for 10 min, after which HA-TAK1 was immunoprecipitated and assayed for kinase activity as described in Fig. 1C. Activity is expressed as fold increase relative to that of HA-TAK1 from unstimulated MYC-TAB1–negative cells. Data are means ± SEM from at least three experiments (upper panel). HA-TAK1 did not phosphorylate KN–p38 (MPK2) directly (*2*). (Middle panel) Autoradiograph showing the phosphorylation of KN–p38 (MPK2). (Lower panel) Immunoblot analysis of each immunoprecipitate with monoclonal antibody 12CA5 to HA demonstrated that approximately the same amount of TAK1-HA was recovered in each sample. Data in middle and lower panels are from a typical experiment.

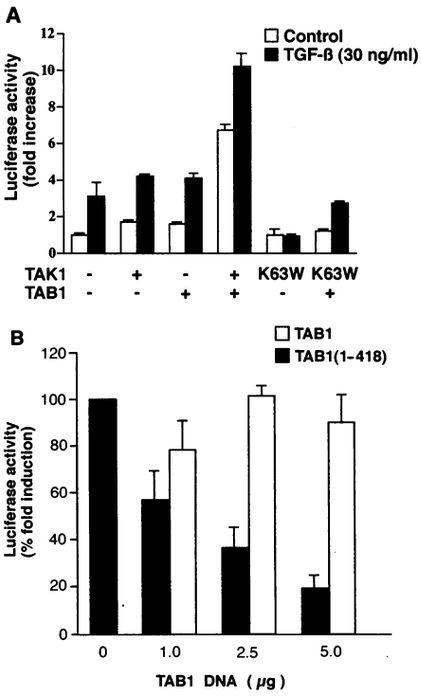

Fig. 4. Effect of TAB1 on TGF-β signaling. (**A**) Effect of TAB1 expression on activation of the PAI1 gene promoter. Mv1Lu cells were transiently transfected with the reporter plasmid p800neoLUC and the indicated plasmids (*14*) by the calcium phosphate method as described (*20*). Cells were subsequently incubated in the absence or presence of human TGF-β1 (30 ng/ml) for 20 hours, after which extracts were prepared and assayed for luciferase activity (*21*). Activities were normalized on the basis of β-galactosidase expression from the pXeX-β-Gal vector (*22*) and are expressed as fold increase relative to unstimulated cells transfected with pEF vector. K63W indicates the catalytically inactive TAK1(K63W). All transfections and luciferase assays were performed in triplicate at least five times. Data are means ± SEM of triplicate determinations from a representative experiment. (**B**) Dose-response effect of TAB1(1–418) on TGF-β–induced gene expression. Mv1Lu cells were transiently transfected with p800neoLUC and the indicated amounts of expression vector encoding full-length TAB1 or TAB1(1–418) (*23*), supplemented to a total of 5 μg of DNA with the pEF control vector. Cells were then incubated with or without TGF-β1 (30 ng/ml) for 20 hours, and cell lysates were assayed for luciferase activity. Activity was expressed as a percentage of the fold induction obtained with TGF-β in control cells transfected with pEF. All transfections and luciferase assays were performed in triplicate at least three times. Data are means ± SEM of triplicate determinations from a representative experiment.

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5. The pLexA-TAK1ΔN plasmid comprises the TAK1ΔN coding sequence (TAK1 residues 21 to 579) (*2*) inserted in frame into pBTM116 [A. B. Vojtek, S. M. Hollenberg, J. A. Cooper, *Cell* **74**, 205 (1993)]. The yeast two-hybrid system was used to identify proteins encoded by a human brain cDNA library that interact with TAK1ΔN. The two hybrids were expressed in a *Saccharomyces cerevisiae* strain L40 (*LYS2::lexA-HIS3*) that contains an integrated reporter construct in which a binding site for the LexA protein was placed upstream of the yeast *HIS3* coding region. If the two hybrid proteins interact, then transactivation of the reporter construct occurs and the yeast cells can grow in the absence of histidine (SC-His). The LexA-TAK1ΔN fusion protein alone induced sufficient *HIS3* expression to allow growth in the absence of exogenous histidine. However, histidine auxotrophy could be reestablished by growing cells in the presence of 40 mM 3-aminotriozole, a chemical inhibitor of the *HIS3* product, imidazole glycerol dehydrogenase [G. M. Kishore and D. M. Shah, *Annu. Rev. Biochem.* **57**, 627 (1988)].
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7. The pheromone-stimulated MAPK pathway consists of the Ste11p, Ste7p, and either Fus3p or Kss1p kinases, which correspond to MAPKKK, MAPKK, and MAPK, respectively. These yeast protein kinases act sequentially to transmit a signal to the transcription factor Ste12p, which activates transcription of mating-specific genes such as *FUS1* [I. Herskowitz, *Cell* **80**, 187 (1995); D. E. Levin and B. Errede, *Curr. Opin. Cell Biol.* **7**, 197 (1995); J. Schultz, B. Ferguson, G. F. Sprague Jr., *Curr. Opin. Genet. Dev.* **5**, 31 (1995)]. The *FUS1p::HIS3* reporter gene, which comprises the *FUS1* upstream activation sequence joined to the *HIS3* open reading frame, allows signal activity in a *his3Δ FUS1p::HIS3* strain to be monitored by the ability of cells to grow on SC-His medium (His phenotype). A *his3Δ ste11Δ FUS1p::HIS3 STE7^{P368}* (Pro substitution at Ser^{368}) strain has a His$^-$ phenotype (*6*). Expression of mammalian TAK1ΔN, but not TAK1, in this strain confers a His$^+$ phenotype (*2*). Thus, the activated form of TAK1 can substitute for Ste11p activity in a Ste7^{P368}-dependent manner.
8. The DNA sequence that encodes the HA epitope recognized by the monoclonal antibody 12CA5 was attached in frame to the DNA encoding the COOH termini of TAK1 and TAK1(K63W) by the polymerase chain reaction (PCR). All constructs were expressed from the *TDH3* promoter. The TAB1 expression plasmid, pGAP-HTH9M, encodes the COOH-terminal 68 amino acids of TAB1. The coding sequence for the COOH-terminal 68 amino acids of TAB1 was amplified by PCR with a 5′ primer (5′-GAGAATTCATGCGGCAAAGC-3′) incorporating an Eco RI site and an ATG codon, and a 3′ primer (5′-GGGTCGACTACGGTGC-3′) incorporating a Sal I site. A 240–base pair Eco RI–Sal I fragment generated by PCR was inserted into the Eco RI–Sal I gap of YEpGAP112, a multicopy *TRP1* plasmid that contains the *TDH3* promoter [H. Banno *et al.*, *Mol. Cell. Biol.* **13**, 4745 (1993)], to generate pGAP-HTH9M.
9. The truncated TAB1 cDNA obtained from the yeast two-hybrid system was used as a probe to screen a human kidney cDNA library (Clontech). The 5′ end of TAB1 mRNA was determined by 5′ rapid amplification of cDNA ends with 5′-RACE-Ready cDNA (Clontech). The nucleotide sequence (CCAAGATGG) at the putative NH_2-terminal end of the coding sequence corresponds to the Kozak consensus [M. Kozak, *J. Cell Biol.* **108**, 229 (1989)], and there are no upstream ATG codons.
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11. The full-length TAB1 cDNA was subcloned into the pCS2MT vector [D. L. Turner and H. Weintraub, *Genes Dev.* **8**, 1434 (1994)], which encodes six copies of the MYC epitope (LEQKLISEEDLN) (*16*) recognized by the monoclonal antibody 9E10. In the resulting plasmid, pCS2MT-TAB1, DNA encoding the MYC epitope tag was attached in frame to the DNA sequence corresponding to the NH_2-terminus of TAB1. The pCS2MT-TAB1 plasmid was digested with Bam HI and Xba I, and the resulting fragment was isolated and inserted into the Eco RI–Xba I sites of the mammalian expression vector pEF, in which expression is controlled by the human elongation factor 1α (EF1α) gene promoter.
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14. The TAB1 expression plasmid, pEF-TAB1, contains the full-length TAB1 coding sequence under the control of the EF1α gene promoter. This plasmid was generated by cleaving the pEF vector with Eco RI and inserting the Eco RI fragment from pBS-TAB1, which itself was generated by subcloning of the TAB1 cDNA into the Eco RI site of pBS.
15. K. Yamaguchi, K. Irie, K. Matsumoto, unpublished data.
16. Abbreviations for the amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
17. Yeast strain L40 was cotransformed with (i) an expression vector encoding full-length TAK1 or a deletion construct fused to the LexA DNA binding domain and (ii) pGAD-TAB1 or pGAD-TAB2. Interaction between fusion proteins expressed by the specified plasmids was demonstrated by the ability of the yeast cells to grow on plates of SC−His containing 40 mM 3-aminotriozole. Each deletion construct of TAK1 was generated from the full-length TAK1.
18. Strain SY1984-P is SY1984 (*his3Δ ste11Δ FUS1p::HIS3*) transformed with plasmid pNC318-P368, which contains the *STE7^{P368}* allele under the control of the *CYC1* promoter (*6*). Plasmids pNV11-HU11 and pNV11-HU11F express, under the control of the *TDH3* promoter, TAK1ΔN and full-length TAK1, respectively (*2*).
19. Yeast cells (60-ml culture) were grown to an optical density at 600 nm of 0.8. Cell extracts were prepared with a lysis buffer as described (*6*) and centrifuged for 30 min at 100,000*g*. The supernatant was subjected to immunoprecipitation with antibodies to HA. Briefly, a portion (300 μl) of the supernatant was mixed with 2 μl of antibodies and 90 μl of protein A–Sepharose (Pharmacia), and the immune complex was washed three times with lysis buffer and assayed for kinase activity (*2*). Immunoblot analysis of each immunoprecipitate with monoclonal antibody 12CA5 to HA demonstrated that approximately the same amount of TAK1-HA or TAK1(K63W)-HA was recovered in each sample, indicating that TAB1 expression did not affect the amount of TAK1 expressed.
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22. Transfection efficiency was normalized by cotransfection with the pXeX-β-Gal vector [A. D. Johnson and P. A. Krieg, *Gene* **147**, 223 (1994)] in all luciferase reporter experiments. The β-galatosidase assay was performed as described by the manufacturer (Clontech) with the cell lysates prepared for luciferase assay.
23. A 1.3-kb Eco RI–Hinc II fragment of pBS-TAB1 (*14*) that encodes amino acids 1 to 418 of TAB1 was subcloned into the pKT10 vector to generate pKT10-TAB1(1–418). The pEF-TAB1(1–418) plasmid was generated by cleaving the pEF vector with Eco RI and Sal I and inserting the Eco RI–Sal I fragment from pKT10-TAB1(1–418).
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Requirement for Cholinergic Synaptic Transmission in the Propagation of Spontaneous Retinal Waves

Marla B. Feller,* David P. Wellis, David Stellwagen, Frank S. Werblin, Carla J. Shatz

Highly correlated neural activity in the form of spontaneous waves of action potentials is present in the developing retina weeks before vision. Optical imaging revealed that these waves consist of spatially restricted domains of activity that form a mosaic pattern over the entire retinal ganglion cell layer. Whole-cell recordings indicate that wave generation requires synaptic activation of neuronal nicotinic acetylcholine receptors on ganglion cells. The only cholinergic cells in these immature retinas are a uniformly distributed, bistratified population of amacrine cells, as assessed by antibodies to choline acetyltransferase. The results indicate that the major source of synaptic input to retinal ganglion cells is a system of cholinergic amacrine cells, whose activity is required for wave propagation in the developing retina.

Spontaneous activity generated in immature circuits is present in various regions of the central nervous system (*1–3*) and participates in the development and differentiation of synaptic circuitry (*4*). Long before the onset of vision, mammalian retinal ganglion cells exhibit spontaneous, periodic bursts of action potentials (*5–7*). At this early stage of development, blockade of neural activity prevents the segregation of retinal ganglion cell axons into appropriate eye-specific layers within their thalamic target nucleus (*8*), and modification of retinal activity at later ages alters the development of retinal ganglion cell dendrites and receptive fields (*9*).

The spatial pattern of activity among neighboring ganglion cells resembles waves that periodically propagate across the ganglion cell layer (*6, 10*). To investigate the

Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720–3200 USA.

*To whom correspondence should be addressed at 221 Life Sciences Addition, University of California, Berkeley, CA 94720–3200, USA. E-mail: marla@violet.berkeley.edu