We have previously characterized two murine cAMP-dependent protein kinase catalytic subunit genes, Ca and Cβ. Targeted disruption of the Cβ1 promoter revealed two splice variants of the Cβ catalytic subunit gene (designated Cβ2 and Cβ3) that continue to be expressed. These variants arise from unique promoters and are brain-specific. Cβ2 is expressed in several discrete areas in the limbic system. These include the lateral septum, the bed nucleus of the stria terminalis, the ventral medial hypothalamus, and the amygdala. In the neocortex, expression is highest in cortical areas such as the prefrontal and insular cortex that are associated with limbic structures. By contrast, Cβ1 is most highly expressed in the cortex and hippocampus and is also present in all non-neuronal tissues examined. Cβ3 is expressed at very low levels with wide distribution throughout the brain. Both the Cβ2 and Cβ3 variants are enzymatically active and induce gene expression in transient transfections with a cAMP response element-reporter construct. This activity is inhibited by protein kinase A regulatory subunits, the protein kinase inhibitor, and the chemical inhibitor H-89. We also demonstrate that Cβ1 is myristoylated at the amino terminus like the Ca isoform, but neither Cβ2 nor Cβ3 is myristoylated. The discrete expression of Cβ variants in the brain suggests specific functional roles in neuronal signaling.

Cyclic AMP-dependent protein kinase (protein kinase A (PKA)) has been shown to be the principal mediator of cellular responses to cAMP in animal cells. The inactive PKA holoenzyme exists as a heterotetramer of two regulatory (R) and two catalytic (C) subunits. The C subunit family consists of two characterized isoforms (Ca and Cβ) that have been described in murine (1), bovine (2), human (3), and porcine (4) tissues. The Ca isoform is expressed ubiquitously, whereas Cβ, although found in all tissues examined, is most highly expressed in the brain (5). A third isoform (Cγ) is found only in primates and is expressed from a processed gene in the testis (3). The murine genome also contains a processed pseudogene related to Ca, but this pseudogene is not transcribed (6).

Although the Ca and Cβ isoforms are 91% identical in amino acid sequence, recent work has revealed distinct biochemical properties that suggest unique functions (7). Ca exhibited a 3–5-fold lower K_m for certain peptide substrates and a 3-fold lower IC_50 for inhibition by PKI or RIIα subunits than did Cβ. In addition, holoenzyme containing RIIα and Cβ is 5-fold more sensitive to activation by cAMP and has a higher basal activity in COS-1 cells than does holoenzyme containing Ca.

The Ca subunit of PKA was one of the first examples of an N-myristoylprotein to be described (8). In the case of members of the Src family of protein-tyrosine kinases, this modification is necessary for targeting the protein to membranes and for normal function (9, 10). N-Myristoylation of Ca does not appear to serve a unique membrane targeting function as the protein is found in many cellular compartments. We have also demonstrated previously that a mutant form of Ca that is not myristoylated has the ability to induce gene expression and modulate steroidogenesis (11).

A targeted disruption of the Cβ gene was created in which the promoter and translational start site were deleted, and mice carrying this mutation lacked the Cβ protein that we had previously characterized (12). We have designated this previously described isoform Cβ1. Mice that lack Cβ1 exhibit a decrease in hippocampal long-term potentiation in the Schaffer collateral-CA1 pathway. In addition, the Cβ1 mutant mice lack both long-term depression and depotentiation in the Schaffer collateral-CA1 pathway. We have also shown that these mice lack long-term potentiation in the mossy fiber-CA3 pathway (13). However, Northern analysis of tissues from Cβ1 knockout mice showed that the Cβ transcript was eliminated from all tissues of the Cβ1 mutants except the brain, where a unique transcript remained. Western blots of brain regions probed with a Cβ antibody revealed a slightly more rapidly migrating band that remained in the knockout mice.

In this paper, we describe two novel splice variants of the murine Cβ gene. These two Cβ variants (designated Cβ2 and Cβ3) diverge from the original Cβ1 sequence at the amino terminus and arise from the use of alternate promoters. Both the Cβ2 and Cβ3 variants are brain-specific and encode functional catalytic subunits that interact with R subunits and PKI. In contrast to the previously studied Ca and Cβ1 proteins, Cβ2 and Cβ3 are not myristoylated at the amino terminus. The Cβ2 transcript is most highly expressed in limbic areas of the brain, whereas Cβ1 is highly expressed in the hippocampus and more diffusely throughout the brain. Expression of Cβ3 is very low and appears to be widely distributed in brain regions.
EXPERIMENTAL PROCEDURES

Oligonucleotide Probes—Oligonucleotides were made to correspond to unique sequences from the first exons of the three Cβ variants. Sequences are as follows: Cβ1, 5'-AAGAAGGGACACGGAATGGAAGGC-3'; Cβ2, 5'-GACACATGGCCTGTCATCATGAAT-3'; and Cβ3, 5'-CCGGTGGATCACACAGGTTG-3'. In addition, oligonucleotides were generated corresponding to common sequence found in exons 2 and 3 of the cDNA probe for mouse exon 2, 5'-GACACGACTCTCAGCTCCTCGTT-3' and exon 3, 5'-AGATAAGGTGCGGCTTGG-3'. These oligonucleotides were end-labeled with [32P]dATP and used to probe Southern blots of genomic clones isolated in our initial characterization of Cβ (14).

Sequencing—Positive cDNA clones identified by screening a mouse brain cDNA library were sequenced using Taq Dye Primer (Applied Biosystems). These were aligned with known Cβ1 sequence and used to generate the cDNA probe for mouse exon 3. A 3.2-kilobase fragment from clone a15, which contains exons 1 of Cβ1 and Cβ3, was subcloned into pUC19. Automated sequencing was performed beginning with pUC19 forward and reverse sequencing primers followed by Taq DyeDeoxy Terminator (Applied Biosystems) sequencing using internal primers designed from previous sequence.

Construction of Riboprobe Vectors—Vectors to produce RNA probes for Northern blotting and in situ hybridization analysis were prepared by PCR from genomic clones, following by subcloning into pBluescript KS-.

To generate a Cβ1 fragment of 71 nucleotides with 5'-EcoRI and 3'-Smal restriction sites, PCR primers 5'-GTTACAACTGCTCTCGTGGGCTATCG-3' and 5'-CTACTGAGTACACGGCTTCCTACCTGCTTGCC-3' were used. For production of a Cβ2 fragment of 291 nucleotides with 5'-EcoRI and 3'-Sacl site, PCR primers 5'-GACACGACTCTCAGCTCCTCGTT-3' and 5'-GAACACGGGCAAAGAGCTC-3' were used. A 1.16-kilobase fragment from Cβ3 was made by PCR with 5'-EcoRI and 3'-Sacl site using primers 5'-GTTAAGAGGTTCCCGGATGCAGTTGG-3' and 5'-GAAATGGCCGACACACTTGTTGG-3'. Antisense RNA probes were generated by linearizing at the EcoRI site and transcribing with T7 RNA polymerase. Probes were isolated on a Sephadex G-50 column, followed by ethanol precipitation.

Northern Blots—Total RNA was isolated from mouse tissues using the guanidine-HCl method. Poly(A)+ RNA was selected using oligo(dT)-cellulose affinity chromatography. RNA samples were electrophoresed on 1.2% agarose denaturing gels, transferred to Hybond-N membranes (Amersham Corp.), UV-cross-linked, and stained with methylene blue to visualize 28 S and 18 S ribosomal RNA bands. Blots were hybridized at 63 °C in hybridization buffer (50% formamide, 5× Denhardt's solution in 50 mM sodium citrate, 37 °C) for 30 min, washed once at room temperature, and washed twice (at 45 °C for Cβ1 and Cβ3) and at 60 °C for Cβ2 in 0.1× SSC. Blots were then washed at room temperature in 1× SSC (150 mM NaCl and 15 mM sodium citrate, pH 7.0). Slides were then treated with 20 μg/ml ribonuclease (Promega) at 37 °C for 30 min and washed three times (at 45 °C for Cβ1 and Cβ3 and at 60 °C for Cβ2) in 0.1× SSC. Slides were hybridized in a graded series of ethanol containing ammonium acetate and air-dried. Slides were assayed on a Molecular Dynamics (Sunnyvale, CA) model ALF Express II gel documentation system.

RESULTS

Genomic Structure of Cβ—A mouse brain cDNA library was screened with a cDNA fragment corresponding to the 5'-end of the previously described Cβ cDNA (14). Positive clones were isolated and sequenced. Two novel Cβ cDNAs were found that diverged from the original Cβ sequence and had unique exon 1 sequences. Oligonucleotides corresponding to unique sequences within exons 1 of the Cβ variants were used to screen a panel of mouse genomic Cβ clones in a Chiron 4A that had been previously isolated (14). Two of these clones, λl0 and λl5, hybridized to one or more of these oligonucleotides. These λ clones were then mapped with a panel of restriction endonucleases and subjected to Southern blot analysis. In addition to the oligonucleotides specific for exons 1 of Cβ1, Cβ2, and Cβ3, oligonucleotides were made to correspond to common sequence in exons 2 and 3. Blots of λl0 and λl5 were probed with these oligonucleotides, and exon 1 of Cβ1 was located in λl0. Exons 1 of Cβ2 and Cβ3 along with exons 2 and 3 were localized in λl5 as shown in Fig. 1.

Labeled probes were made from EcoRI fragments of the two

Brain-specific Variants of PKA Cβ 29561
FIG. 1. Cβ genomic structure. Genomic clones 1A10 and 1A15 were mapped with restriction enzymes as indicated. Southern blots were probed with oligonucleotides to exons 1 of Cβ1, Cβ2, and Cβ3 and to the common Cβ exons 2 and 3. A 3.4-kilobase (kb) EcoRI fragment of 1A5 that contains cDNA sequence for exons 1 of Cβ2 and Cβ3 was sequenced, and translational start sites were located. Sequences used to produce RNA probes are indicated by horizontal bars. The exon sequences are boxed, and the start sites of translation are indicated.

A clones. Probes were then hybridized with Southern blots of the other A clone. No cross-hybridization was detected, indicating that 1A10 and 1A15 do not overlap. This leaves a distance of at least 9 kilobases between Cβ1 exon 1 and Cβ3 exon 1. The 3.4-kilobase EcoRI fragment of 1A5 was sequenced, and the positions of the exons are indicated (Fig. 1). The restriction site indicated are those mapped in 1A15 and confirmed when the fragment was sequenced.

Sequence Comparison of Cβ Splice Variants—Fig. 2 shows the sequence from the XbaI site to the end of Cβ2 exon 1. Transcriptional (arrows) and translational (Met) start sites are indicated for Cβ3 and Cβ2. Sequences of the first exons of the three Cβ variants (Fig. 2B) are aligned at the splice junction of the common exon 2. Cβ2 has only two amino acids in exon 1, and Cβ3 has three amino acids compared with 15 amino acids in exon 1 of Cβ1. Cβ3 also has a T instead of a G in the first position of codon 4, which changes the first amino acid of exon 2 from valine to leucine. Cβ1 and Cβ3 have the N-terminal glycine residue that can provide a substrate for myristoylation, whereas Cβ2 lacks this residue.

3H-Myristic Acid Labeling of Cβ Splice Variants—Mouse NIH 3T3 cells that overexpress each of the three Cβ splice variants were cultured as described under "Experimental Procedures." These cultures were exposed to 50 µCi ZnSO4 to induce the metallothionein promoter-driven expression vectors. Western analysis using anti-C subunit antisera is shown in Fig. 3B. In the absence of ZnSO4 induction, the endogenous level of Cα and Cβ1 was observed as a single comigrating band. A previously described cell line overexpressing Cα was also included as a positive control (11). In addition to induction of the expression vectors, Fig. 3B shows bands for Cβ2 and Cβ3 migrating more rapidly than those for Cβ1, consistent with our predicted translational start sites and corresponding to the bands observed in brain extracts. Extracts from these cultures were run in parallel, and 3H-myristic acid incorporation was detected using fluorography (Fig. 3A). Myristoylation occurred on Cα and Cβ1, but neither Cβ2 nor Cβ3 was a target for N-myristoylation.

Tissue-specific Expression Patterns of the Three Cβ Variants—Northern blot analysis of a panel of tissues isolated from both wild-type and Cβ1 knockout mice revealed different patterns of expression for the three variants of Cβ (Fig. 4). Cβ1 mRNA was found in all tissues examined, but the magnitude of expression varied, with the highest expression in the brain and a very faint signal in the testes. Cβ2 had strong expression in the brain and was found in no other tissue examined. Cβ3 was also found only in the brain, but at very low levels. It was necessary to isolate poly(A)+ RNA to detect a clear Cβ3 signal. There was no compensatory increase in either Cβ2 or Cβ3 mRNA transcripts in brains from Cβ1 mutant mice.

A peptide-specific antibody capable of recognizing all Cβ
variants was used in Western blotting to demonstrate the unique expression of Cβ2/3 variants in the brain (Fig. 4D). We could not distinguish between Cβ2 and Cβ3 because they comigrated on gels, but the relative abundance of Cβ2 mRNA suggests that Cβ2 protein accounts for nearly all of the lower molecular weight band expressed in the brain.

Functional Testing of Cβ Variants—Transient cotransfection assays were performed to determine whether the two novel Cβ variants produced active C subunit that can regulate expression of a CAMP response element-driven reporter construct. Both the Cβ2 (Fig. 5B) and Cβ3 (Fig. 5C) variants were capable of stimulating the CAMP response element reporter expression to a level similar to that observed for the previously described Cβ1 isoform (Fig. 5A). Each of the three variants was able to bind either type I or II R subunits as evidenced by inhibition of reporter gene transactivation. Activity was restored upon treatment with forskolin, which increases CAMP. Cotransfection with an expression vector for the heat-stable inhibitor of PKA (PKI) inhibited induction in a CAMP-independent manner for all Cβ variants. Thus, Cβ2 and Cβ3 behaved in this assay in a manner consistent with previous studies using Ca or Cβ1.

Many laboratories have relied on the use of specific chemical inhibitors of PKA to implicate this enzyme in regulatory pathways. A recent study demonstrated that the effects of cAMP on dopaminergic regulation of gene expression in developing striatum could not be blocked by the inhibitor H-89 (N-(2-((p-bromocinnamyl)amino)ethyl)-5-isoquinolinesulfonamide HCl), and the authors suggested the possibility that brain-specific isoforms of PKA might exist that are insensitive to H-89 (21). Since Cβ2 and Cβ3 represent novel brain-specific catalytic subunits, we examined their H-89 sensitivity, and as shown in Fig. 5D, we conclude that these Cβ variants are inhibited effectively by H-89. We cannot rule out the possibility that other Cβ or Ca variants exist that exhibit altered H-89 sensitivity.

In Situ Hybridization of Coronal Sections of Mouse Brain—Autoradiographic analysis of cryosections of mouse brain hybridized with Cβ isoform-specific riboprobes revealed very different distributions of the three mRNAs (Fig. 6). The Cβ1 isoform was most highly expressed in the dentate gyrus and the pyramidal cell layers of the hippocampus. An intense signal was also observed in the habenula. A generalized distribution was seen throughout the neocortex. Cβ1 mRNA was also present in the thalamic areas, although no specific nuclei were evident. The same was true for the hypothalamus, with some localization in the dorsal medial and ventral medial hypothalamic nuclei.

Cβ2 showed a very distinct and interesting pattern of expression. In the rostral part of the brain, intense labeling was seen in the prelimbic cortex. A concentration of Cβ2 was also seen in the insular cortex. Cβ2 mRNA was localized to the lateral septum, with the intermediate nucleus showing the most intense labeling. The bed nucleus of the stria terminalis was also strongly labeled with the Cβ2-specific probe. The signal in the more caudal parts of the forebrain also differed from that of Cβ1. The intense hippocampal signal seen with the Cβ1 probe was absent, and only the dentate gyrus expressed Cβ2 mRNA. The neocortex in this medial area of the brain contained a substantial amount of both Cβ1 and Cβ2 mRNAs. The amygdala displayed a prominent Cβ2 signal, with lateral, medial, basolateral, and cortical amygdaloid nuclei evident. There was an absence of Cβ2 hybridization in the thalamic area and a diffuse labeling of the hypothalamus, except for an intense label in the ventral medial hypothalamic nucleus. The signal for Cβ3 was very faint, and its distribution was similar to that of Cβ1. Competition with a 100-fold excess of unlabelled probe indicated that the signals observed are highly specific.

DISCUSSION

We have characterized two novel variants of the murine Cβ gene of PKA that are specifically expressed in the brain. These variants (designated Cβ2 and Cβ3) arise by the use of alternate first exons with distinct transcriptional start sites.

This diversity in the N-terminal exon of PKA genes has been observed in species other than mouse. In Aplysia, a neuronal form of PKA has been shown to have two alternate N termini that can combine with either of two internal cysteines to produce enzymes with different biochemical characteristics (22, 23). PKA C subunits with extended amino termini have also been described in Drosophila (24) and Dictyostelium (25). A cDNA encoding an alternately spliced form of the Cβ subunit has been cloned from bovine heart. This bovine Cβ variant has an alternate first exon that is spliced at the same place as the murine variants (26). Because the murine Cβ splice is in the identical location as in the bovine Cβ variant, our genomic clones were probed with an oligonucleotide made against the bovine sequence. No hybridization of this bovine sequence was seen with our murine genomic clones. However, because our genomic clones do not overlap, the presence of a sequence like
the bovine Cβ variant in the intervening space is possible.

Analysis of the genomic sequence in the region that contains the alternate first exons of Cβ2 and Cβ3 reveals differences between these splice variants and the other PKA C subunits described previously (1). The murine Ca gene and the Cβ isoform have multiple sites of transcriptional initiation and a GC-rich 5′-flanking region typical of many constitutively active genes (14). In contrast, Cβ2 and Cβ3 have a single major transcriptional start site and an AT-rich promoter. Also of note is the presence of short upstream open reading frames in the 5′-leaders of Cβ2 and Cβ3. This structure is characteristic of genes that exhibit translational control (27). Our in situ results demonstrate cell type-specific control at the transcriptional level, but the presence of these upstream open reading frames suggests possible regulation at a post-transcriptional level as well.

Our functional testing reveals that the proteins made from the Cβ2 and Cβ3 sequences are able to activate a cAMP-responsive reporter gene. They are inhibited by R subunits and regain activity upon elevation of cAMP levels. They are also inhibited when PKI is overexpressed or the PKA-selective chemical inhibitor H-89 is present. There have been suggestions in the literature that neural C subunit isoforms might exist that could be resistant to typical PKA inhibitors (21). Our in situ results demonstrate cell type-specific control at the transcriptional level, but the presence of these upstream open reading frames suggests possible regulation at a post-transcriptional level as well.

Functional analysis of Cβ variants. JEG-3 cells were transiently transfected with a cAMP-responsive reporter (α188-luciferase) and an RSV-β-galactosidase construct to control for transfection efficiency. Expression vectors for Cβ1 (A), Cβ2 (B), and Cβ3 (C) catalytic subunits were cotransfected along with expression vectors for RI or RII regulatory subunits or the heat-stable PKA inhibitor (PKI). Cells were treated with vehicle or 30 μM forskolin (fsk) for 6 h, harvested, and assayed for luciferase and β-galactosidase activities. Cells overexpressing each of the C subunits were treated for 8 h with the indicated concentrations of the PKA-selective chemical inhibitor H-89 before measuring luciferase induction (D).

Brain-specific Variants of PKA Cβ

In situ hybridization analysis of Cβ variants in mouse brain. Wild-type mouse brain cryosections (20 μm) were hybridized to RNA probes specific for Cβ1, Cβ2, and Cβ3. Hybridizations were carried out overnight at 55 °C (Cβ1 and Cβ3) or 60 °C (Cβ2). Locations of coronal sections are marked on the sagittal section. Coronal sections are shown as follows: row a, bregma 1.42 mm; row b, bregma 0.62 mm; row c, bregma 0.14 mm; row d, bregma -1.22 mm; row e, bregma -1.46 mm; and row f, bregma -2.92 mm (31). Control sections (bregma -1.22 mm) were hybridized with a 100-fold excess of the corresponding unlabeled probe plus the labeled Cβ1, Cβ2, or Cβ3-specific probe. ACo, anterior cortical amygdaloid nucleus; BLA, basolateral amygdaloid nucleus; BMA, basomedial amygdaloid nucleus, anterior; BST, bed nucleus stria terminalis; CA1, CA1 pyramidal layer of the hippocampus; CA3, CA3 pyramidal layer of the hippocampus; ChPl, choroid plexus; DG, dentate gyrus; Hb, habenula; IMLF, interstitial nucleus, medial longitudinal fasciculus; Ins, insular cortex; MeA, medial amygdaloid nucleus; MP, medial mamillary nucleus, posterior; Pir, piriform cortex; PMCo, posterior medial cortical amygdaloid nucleus; PMV, premamillary nucleus, ventral; Prl, prelimbic cortex; LSD, lateral septum, dorsal; LSV, lateral septum, ventral; TM, tuberomamillary nucleus; VMH, ventral medial hypothalamic nucleus.
Brain-specific Variants of PKA Cb

29565

tively charged residues in the enzymatic cleft of N-myristoyl-
transferase. In addition, the bulky aromatic residue at position
6 is likely a stearic hindrance.

We found Cb2 and Cb3 mRNAs to be expressed only in the
brain. Cb2 has a high level of expression, approximately equi-

tent to that of Cb1 in preparations of whole brain. Cb3 is
expressed at a low level in total brain RNA, and it does not
appear to be regionally localized by in situ hybridization. Nei-
	her Cb2 nor Cb3 mRNA appears to compensate for the loss of
Cb1 mRNA in null mutants. This was assessed both by North-
ern blots prepared from whole brain and by in situ hybridiza-
tion experiments on wild-type and Cb1 mutant brains (data not
shown). We also observed no increase in the lower molecular
weight Cb band on Western blots from Cb1 mutant mice, indi-
cating no compensation at the protein level (12).

The differential distribution of Cb1 and Cb2 mRNAs in dis-
crete areas of the mouse brain is intriguing. The appearance of
prominent expression of Cb1 in the hippocampus was expected
and is consistent with the defects seen in late-phase long-term
potentiation and long-term depression in the Schaffer collater-
al-CA1 synapse (12). Previous work (5) has shown that Cb is
expressed in these same regions, but is apparently unable to
functionally compensate for Cb1. It is possible that Cb may not
be expressed in the proper cells or in the correct sub-

The neocortex is labeled throughout by the Cb2 probe, with
the prelimbic cortex and insular cortex being particularly in-
tense. This is of interest, as these brain regions have been
implicated in the pathophysiology of schizophrenia and are rich
in D1 receptors that act by elevating cAMP (30). The septal
region has strong signals for Cb2 in the lateral septal nucleus
and the bed nucleus of the stria terminalis. In the more caudal
aspect of the forebrain region, we see a strong Cb2 signal in the
ventral medial hypothalamic nucleus. This nucleus has been
implicated in alterations in feeding behavior and aggression.
The amygdala, like the hypothalamus, is an integrator of input
from other limbic structures and is labeled with the Cb2 probe
in the cortical, medial, basal, and basolateral nuclei. We can
only speculate as to the function of Cb2 in these areas. The
amygdala and its homologous rostral extension, the bed nu-

cleus of the stria terminalis, have some of the heaviest innera-
vation by dopaminergic neurons in the brain, where Cb2 could
be activated via D1 dopamine receptor binding. There are nu-
merous interconnections among these regions, and they are
believed to be involved in learning and memory, emotion, and

more primitive aspects of behavior such as flight from danger
or pain avoidance. The functional significance of these variants
awaits targeted disruption of Cb2 and Cb3 and their effects on
neural function and behavior.

Acknowledgments—We acknowledge Paul Amieux for helpful dis-
cussions during preparation of the manuscript and Jyoti Watters for as-
sistance with in situ hybridization. We are also grateful to Rejean
Idzerda for helpful experimental advice and comments on the
manuscript.

REFERENCES

1. Uhler, M. D., Chrivia, J. C., and McKnight, G. S. (1986) J. Biol. Chem.
261, 15360–15363
2. Showers, M. O., and Maurer, R. A. (1986) J. Biol. Chem. 261, 16288–16291
3. Beebe, S. J., Oyen, O., Sandberg, M., Froyss, A., Hansson, V., and Jahn, T.
(1990) Mol. Endocrinol. 4, 465–475
4. Advani, S. R., Schwartz, M., Showers, M. O., Maurer, R. A., and Hemmings,
B. A. (1987) Eur. J. Biochem. 167, 221–226
5. Cadd, G., and McKnight, G. S. (1989) Neuron 3, 71–79
6. Cummings, D. E., Edelhoch, S., Diathece, C. M., and McKnight, G. S. (1994)
Mamm. Genome 5, 701–706
7. Gamm, D., M. Baude, E. J., and Uhler, M. D. (1996) J. Biol. Chem. 271,
15736–15742
8. Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C., and Titani, K. (1982) Proc.
Nat. Acad. Sci. U. S. A. 79, 6128–6131
9. Gordon, J. I., Duronio, R. J., Rudnick, D. A., Adams, S. P., and Gekel, G. W.
(1991) J. Biol. Chem. 266, 8647–8650
10. Resh, M. D. (1994) Cell 76, 411–413
11. Clegg, C. H., Ran, W., Uhler, M. D., and McKnight, G. S. (1989) J. Biol. Chem.
264, 20140–20146
12. Qi, M., Zhou, M., Skålhegg, B. S., Brandon, E. P., Kandel, E. R., McKnight,
G. S., and Idzerda, R. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1571–1576
13. Huang, Y. Y., Kandel, E. R., Varshavskyy, L., Brandon, E. P., Qi, M., Idzerda,
R. L., McKnight, G. S., and Bourtchouladze, R. (1995) Cell 83, 1211–1222
14. Chrivia, J. C., Uhler, M. D., and McKnight, G. S. (1988) J. Biol. Chem. 263,
5739–5744
15. Uhler, M. D., and McKnight, G. S. (1987) J. Biol. Chem. 262, 15202–15207
16. Correll, L. A., Woodford, T. A., Corbin, J. D., Mellon, P. L., and McKnight, G. S.
(1989) J. Biol. Chem. 264, 16672–16679
17. Otten, A. D., and McKnight, G. S. (1989) J. Biol. Chem. 264, 20255–20260
18. Clegg, C. H., Correll, L. A., Cadd, G. G., and McKnight, G. S. (1987) J. Biol.
Chem. 262, 13111–13119
19. Thomas, J., Van Patten, S. M., Howard, P., Day, K. H., Mitchell, R. D., Osick,
T., Trewella, J., Walsh, D. A., and Maurer, R. A. (1991) J. Biol. Chem. 266,
10906–10911
20. van Doren, K., Hanahan, D., and Gluzman, Y. (1984) J. Virol. 50, 606–614
21. Liu, F. C., Takahashi, H., McKay, R. D., and Graybiel, A. M. (1995) J. Neurosci.
15, 2367–2384
22. Cheley, S., and Bayliss, H. (1991) Biochemistry 30, 10246–10255
23. Beushausen, S., Lee, E., Walker, B., and Bayliss, H. (1992) Proc. Natl. Acad.
Sci. U. S. A. 89, 1641–1645
24. McTavish, A., Li, W., and Kalderon, D. (1995) Genetics 141, 1507–1529
25. Anjard, C., Etchebehere, L., Pinaud, S., Veron, M., and Reymond, C. D. (1993)
Biochemistry 32, 9532–9538
26. Wiemann, S., Kinzel, V., and Pyerin, W. (1991) J. Biol. Chem. 266, 5140–5146
27. Gabelle, A. P., and Morris, D. B. (1994) Trends Biochem. Sci. 19, 159–164
28. Towler, D. A., Gordon, J. I., Adams, S. P., and Glaser, L. (1988) Annu. Rev.
Biochem. 57, 69–99
29. Yamamoto, W., McGhine, M. L., and Taylor, S. S. (1993) J. Biol. Chem. 268,
2348–2352
30. Okubo, Y., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., Someya, Y., Sassa,
T., Suda, Y., Matsushima, E., Iyo, M., Tatena, Y., and Toru, M. (1997) Nature
385, 634–636
31. Franklin, K. B. J., and Paxinos, G. (1997) The Mouse Brain in Stereotaxic
Coordinates, Academic Press, New York

2 C. R. Guthrie, B. Skålhegg, and G. S. McKnight, unpublished observations.