Cloning and Sequence Analysis of cDNA for a Neuronal Cell Membrane Antigen, HPC-1*

Akihiro Ioue§, Kunihiko Obata‡, and Kimio Akagawa‡‡

From the §Department of Physiological Sciences, School of Life Sciences, The Graduate University for Advanced Studies and the Laboratory of Neurochemistry, Department of Molecular Physiology, National Institute for Physiological Sciences, Okazaki, Aichi 444, Japan

(Received for publication, December 3, 1991)

A monoclonal antibody (mAb), HPC-1, labels the plasma membrane of the amacrine cell soma and inner plexiform layer in rat retinas and other central neurons. HPC-1 antigen recognizes several proteins of about 35 kDa. In this study, an HPC-1 positive cDNA, HPC-113, was isolated from a Xgtll cDNA library of the rat hippocampus. HPC-113 had the 594-base pair nucleotide sequence in an open reading frame and the calculated molecular mass of the deduced amino acid sequence (298 residues) was 33,989 Da, implying that HPC-113 contains almost the full-length coding region of HPC-1 antigen mRNA. Sequence analysis suggested that HPC-1 antigen is an integrated membrane protein revealing the characteristic α-helical structure with periodical heptad repeats usually seen in proteins with coiled-coil structures. Although the entire amino acid sequence did not show significant homology to any proteins so far known, a few local sequences in the possible extracellular domain of the HPC-1 antigen molecule had notable homology to some partial sequences in the laminin B1 chain. These sequences of laminin are included in the portion which has neurite outgrowth and/or survival promoting activity. The HPC-1 gene was transcribed in nerve tissues much more predominantly than in non-neuronal tissues. Thus, HPC-1 antigen(s) was confined to be a newly identified neuronal cell membrane protein(s) localized in a subpopulation of neurons.

The nervous system consists of highly differentiated and heterogeneous cell types. The retina is an excellent model for studying neuronal pattern formation or neural networks, since its tissue architecture is simple, and there are only a few cell types with well defined physiological roles (1). However it has been difficult to study the molecular basis underlying the retinal structures by histological or biochemical methods.

The abbreviations used are: mAb, monoclonal antibody; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; kb, kilobase pair(s); bp, kilobase(s); PCR, polymerase chain reaction; bp, base pair(s).

*This work was supported in part by a grant from the Daiko Foundation (to A. I.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D10392.

†Fellow of the Japan Society for the Promotion of Science for Japanese Junior Scientists.

‡To whom all correspondence and reprint requests should be addressed: Laboratory of Neurochemistry, National Institute for Physiological Sciences, Okazaki, Aichi 444, Japan. Fax: 81-564-52-7913.

Monoclonal antibodies (mAbs)* recognizing specific antigens of the distinct cell types or layers offer a good approach by which to investigate the characteristics of small amounts of substances in tissues. In this context, a number of cell type specific mAbs for each cell type in retina have been reported (2-11). A monoclonal antibody, designated HPC-1, was produced as one of a series of such mAbs against neuronal membranes of the hippocampus. Since, in adult rat retina, mAb HPC-1 recognized plasma membranes of amacrine cells and inner plexiform layer (9), mAb HPC-1 has been used only for identification of amacrine cells in the retinal monolayer cultures (11-13) and in reaggregate cultures (12, 14, 15). The molecular structure and function of HPC-1 antigen, however, are not understood. Preliminary immunoblotting results indicated that HPC-1 antigen was a 35-kDa protein (9). Developmental studies of rat retinal tissue and reaggregate culture demonstrated that HPC-1 immunoreactivity appeared in postmitotic migrating cells (9).

In the present study, we isolated, identified and analyzed the sequence of a cDNA clone for HPC-1 antigen and demonstrated tissue expression of its mRNA. We further discuss some properties of the deduced amino acid sequence and possible biological functions.

MATERIALS AND METHODS

RNA Isolation—Total cellular RNA was extracted from various tissues of rat by the LiCl/urea lysis and precipitation method (16) with slight modification. Briefly, tissues were homogenized in a lysis solution containing 3 M LiCl, 6 M urea, and RNAs were precipitated overnight at 0 °C. The RNA precipitates were collected by centrifugation, optionally washed with the lysis solution for several samples, redissolved in proteinase K digestion buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5% SDS, and 200 μg/ml proteinase K), and incubated at 37 °C for 4 h. The solution was extracted with the usual organic solutions by the standard method (17) and precipitated by ethanol. Poly(A)-rich RNA was selected using oligo(dT)-cellulose (17).

cDNA Cloning and Sequencing—cDNA was synthesized from rat hippocampus using poly(A)-rich RNA, heated to 70 °C, and immediately cooled to 0 °C, with hexanucleotides random primers using a commercially available system (Amersham). Internal EcoRI sites were methylated, and cDNA was ligated with an EcoRI linker (CCGAATTCGG, Pharmacia), and a Xgtll cDNA library was constructed. Independent recombinant clones, 1 × 10⁶, were screened by the standard method (18, 19). To detect β-galactosidase/HPC-1 antigen fusion proteins, replica nitrocellulose filters from the plate on which the clones were grown (Hybond-C, Amersham) were incubated with mAb HPC-1 at 4 °C for 12 h, washed, and reacted with horse-radish peroxidase-conjugated goat anti-mouse IgG (Cappel). The
peroxidase reaction and detection of positive signals were performed using the Immunostain kit (Konica, Japan) according to the manufacturer's recommendations. We obtained five independent positive clones and the longest one, HPC-113, carrying a 2.1-kbp insert, was provided as a probe for the next screening. RNA blotting and PCR experiments using the longest clone, HPC-113 cDNA, were performed by the method of Henikoff (21) with slight modifications. Single-stranded DNA was rescued and sequenced in both directions by the dideoxy-chain-termination method (22, 23).

RNA Blotting—Poly(A)-rich RNA, 3 μg, isolated from various tissues as described above, was separated on a 1.2% formaldehyde gel and transferred to Genescreen Plus membrane (Du Pont-New England Nuclear) in 10 mM Tris-HCl, pH 7.4, 50% formamide, 1 M NaCl, 100 pg/ml gelatin, 0.2 mM each of [γ-32P]ATP, [γ-32P]GTP, [γ-32P]CTP, and [γ-32P]UTP, and 200 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) at 37 °C for 80 min with an intensifying screen.

RNA Polymerase Chain Reaction Assay—Total RNA (5 μg) prepared from various rat tissues was reverse transcribed using oligo(dT) 

DNA was synthesized from poly(A)-rich RNA of adult rat hippocampus by random priming with hexanucleotides. We used a rabbit reticulocyte lysate pretreated with micrococcal nuclease (Millipore Ltd.). Protein bands on the membranes were stained with amido black. Dilutions of first antibodies were 300 times for HPC-1 antisera and 1,000 times for the antiserum. Positive bands were detected by HRP-conjugated goat anti-mouse (for HPC-1) or anti-rabbit (for antisemur) IgG (Cappel) or Zymed) as second antibodies according to the previous method (26).

In Vitro Transcription and Translation—The 1.94-kb EcoRI/SphI fragment of the HPC-113 insert containing the open reading frame sequence was subcloned into pGEM-4Z (Promega) to produce pG4Z/HPC113TS. The recombinant plasmid (5 μg) was linearized at the HindIII site in the multiple cloning site of pGEM-4Z. This template DNA was treated with proteinase K and transcribed at 37 °C for 2 h in 125 μl of a buffer described in the previous study (27) with slight modifications, in that 2 units/ml of SP6 RNA polymerase (Promega) was used and the capping structure was omitted. The transcript (0.7 μg) was translated in a rabbit reticulocyte lysate pretreated with micrococcal nuclease (Du Pont-New England Nuclear) at 30 °C for 80 min in the presence of [35S]methionine.

Immunohistochemistry—Adult rats were anesthetized with pentobarbital and perfused transcardially with phosphate-buffered saline followed by 4% paraformaldehyde in phosphate-buffered saline. The tissues were dissected out and immersed in 30% sucrose. Cryostat sections of these tissues were collected onto gelatin-coated slides. The sections were incubated with the first antibody at 4 °C for 16 h, washed in phosphate-buffered saline, and reacted with second antibody (goat anti-mouse or rabbit IgG-conjugated with rhodamine (Cappel) as described earlier (11).

Analysis of DNA and Protein Sequences—Nucleotide and amino acid sequences were analyzed with the programs from GENETYX software package (Software Development Co., Japan). Hydrophobicity analysis was conducted with the HYDRO algorithm described by Kyte and Doolittle (28). The secondary structure prediction was carried out with the CHIME program originally developed by Chou and Fasman (29) or the ROBON protein (30). Computer searches of the NBRF-PDB (Release 27) and the SWISS-PROT (Release 15) Protein Sequence Databases for the deduced amino acid sequence were performed by the FAST HOMOLOGY SEARCH program. Amino acid sequences were compared and aligned with the HOMO-GAPP program.

RESULTS

Cloning of HPC-1-positive cDNAs—mAb HPC-1 was a specific marker of amacrine cells in rat retina, staining plasma membranes of these cell somas and inner plexiform layer, in which neural processes of the amacrine cells were extensively distributed. However, mAb HPC-1 was originally produced against the crude membrane fraction of the rat hippocampus (9); therefore, we selected hippocampal tissue as a source of mRNAs for cDNA synthesis to isolate HPC-1-positive cDNA. cDNA was synthesized from poly(A)-rich RNA of adult rat hippocampus by random priming with hexanucleotides. We screened 1 × 106 independent plaques of Λgt11 cDNA library with mAb HPC-1 and subsequently purified five plaques carrying HPC-1 antigenicity. One of them, designated HPC-113, had the longest insert (2.1 kb), and three other clones were of similar length, whereas the remaining two clones, HPC-104, had a shorter insert (0.4 kb). We further constructed another oligo(dT)-primed Λgt10 cDNA library and obtained a longer clone, HPC-202, including a 95-bp poly(A) stretch. Restriction mapping indicated that they had the same pattern in the multiple cloning site of pGEM-4Z. This template DNA was treated with proteinase K and transcribed at 37 °C for 2 h in 125 μl of a buffer described in the previous study (27) with slight modifications, in that 2 units/ml of SP6 RNA polymerase (Promega) was used and the capping structure was omitted. The transcript (0.7 μg) was translated in a rabbit reticulocyte lysate pretreated with micrococcal nuclease (Du Pont-New England Nuclear) at 30 °C for 80 min in the presence of [35S]methionine.

Identification of cDNA Clone for HPC-1 Antigen—To identify the HPC-1-positive cDNA clone, we produced an antisera against the β-galactosidase/HPC-113 fusion protein and compared properties of both mAb HPC-1 and the anti-
serum by immunoblotting and immunohistochemical staining. In whole extracts of bacterial host Y1089 lysogenized with wild type λgt11 phage, native β-galactosidase was a major band of 115 kDa (Fig. 1, lane a), whereas in an extract of Y1089 lysogenized with HPC-113 recombinant Agt11 phage, a band, about 150 kDa, appeared instead of the native β-galactosidase (Fig. 1, lane b). This 150-kDa band was specifically recognized by mAb HPC-1, but no staining was seen in the wild type extract (Fig. 1, lanes c and d). Thus, it was concluded that the 150-kDa band was the β-galactosidase/HPC-113 fusion protein. The 150-kDa band was then electrophoretically purified from the excised gel and used for immunization of rabbits. With several boosters, an antiserum against the fusion protein was raised. In immunohistochemical studies, mAb HPC-1 stained cell membranes of amacrine cells and inner plexiform layer (Fig. 2a) (9), and the antiserum also exhibited the same pattern (Fig. 2b). Fig. 3 shows the results of immunoblotting with mAb HPC-1 and the antiserum. Although it was reported in a previous study that a membrane fraction from these tissues did not react with the antibodies. The antiserum also recognized the same two bands in retina and hippocampus (Fig. 3, lanes c and d), whereas another band with slightly faster mobility (apparently Mr = 34,000) was detected in the cerebral cortex (Fig. 3, lane e). The antiserum reacted more intensely with the lower band in the hippocampus (middle band in the cerebral cortex), unlike mAb, although the reason for this remains unknown.

HPC-113 DNA fragment was transcribed and translated in vitro. In this case, the translation was efficiently initiated at Met11 (see Fig. 6). In a SDS-PAGE (Fig. 3, lane f), a 33,067-Da polypeptide calculated from the cDNA sequence migrated at the same mobility as the lowest band, recognized by the antiserum only in cerebral cortex (Fig. 3, lanes c and g).
using 3 μg of the poly(A)-rich RNA isolated from non-neuronal tissues, i.e., liver, heart, kidney, intestine, or thymus (Fig. 4). There results suggested that the HPC-1 gene was expressed specifically to nerve tissues. The HPC-1 mRNA signal was not detected in retina despite HPC-1 antigen having been immunohistochemically recognized (Fig. 2), indicating that the content of HPC-1 mRNA is considerably low among all mRNAs of the rat retinas.

We further carried out an RNA PCR assay to identify HPC-1 mRNA in the tissues which were negative on the RNA blotting (Fig. 5). PCR was done for cdNA synthesized from total RNAs of rat tissues using the two primers (FP1 and RP3, see "Materials and Methods") which were chosen in order to cover a large part of the coding sequence of HPC-1 cdNA; the amplified DNA fragment from mRNA must be DNA probe generated by another PCR were certainly derived from HPC-1 mRNA, since primers (FP4 and RP4) for probe gene was transcribed predominantly in the neuronal tissues which were chosen in "Materials and Methods") which were chosen in order to cover a large part of the coding sequence of HPC-1 cdNA; the amplified DNA fragment from mRNA must be DNA probe generated by another PCR were certainly derived from HPC-1 mRNA, since primers (FP4 and RP4) for probe gene was transcribed predominantly in the neuronal tissues which were negative on the RNA blotting (Fig. 5). There results suggested that the HPC-1 gene was transcribed predominantly in the neuronal tissues with only weak expression being detected in non-neuronal tissues. The PCR product of non-neuronal tissues, except for the intestine, was slightly longer than that of neuronal tissues.

**Nucleotide and Deduced Amino Acid Sequence of HPC-1 Antigen Molecule**—The nucleotide sequence of the longest cdNA with an appropriate connection (HPC-113 and HPC-202) is shown in Fig. 6. An open reading frame from the EcoRI cloning site of xgt11 continued up to the cytosine (C) at nucleotide number 895, and an in-frame stop codon, TGA, was found at 896–898, followed by a 1326-bp possible 3'-noncoding sequence. One poly(A) addition signal, AATAAA was 19 nucleotides upstream from the poly(A) addition site.

**Fig. 6. Nucleotide and deduced amino acid sequences of rat HPC-1 antigen molecule cloned in HPC-113 and HPC-202.** Nucleotide residues are numbered starting at the next residue of EcoRI linker at the cloning site, and deduced amino acid residues are numbered beginning at the first residue (Gln) in the open reading frame. The candidate for the initiative methionine codon, ATG (boxed, underlined), is boxed in the top of lanes. The single poly(A) addition signal in the 3'-untranslated region is underlined. Two possible N-linked glycosylation sites and the highly hydrophobic sequence are boxed. Two partial amino acid sequences representing similarities with partial lamin B1 chain sequences are indicated as fragment a (a) and fragment b (b) by bold underlines, respectively.

The deduced amino acid sequence of the 894 bp was 298 residues with a calculated molecular mass of 33,989 Da. The shorter positive cdDNA clone, HPC-104, contained G to G415 (416 bp). Accordingly, the epitope for mAb HPC-1 could be to be confined to the sequence of Lys117–Ser160 which is coded by HPC-104.

The hydrophobic profile (Fig. 7) showed a remarkable hydrophobic region in the carboxyl (COOH)-terminal end, Ile776, Gly790. On the amino (NH2)-terminal side out of this region, there were some polar amino acids: Arg272, Arg273, Lys274, Lys275. These were characteristics of membrane spanning regions (28, 32, 33). Considering these results together with the immunohistochemical studies (Fig. 2), the HPC-1 antigen might be an integrated membrane protein. If it is true, a hydrophobic signal sequence would usually be laid in the NH2-terminal portion (34); however, neither a candidate for a signal sequence nor a possible cleavage site is detected in the NH2 terminus (35, 36). On the other hand, a methionine residue, which was a candidate for the initiation site, was seen at number 11 (ATG) and was followed by 287 amino acid residues. In this case, the expected molecular mass was 33,067 Da. Although the sequence just upstream of this ATG (CGAGC ATG) is consistent with the typical consensus sequences for an active eukaryotic translation initiation site, CCA/GGC AU(T)G (37), there is little evidence that this ATG is the real initiation site. As it appeared that other cdDNA clones with longer 5' sequences might be present, we
re-screened the randomly primed and oligo(dT)-primed cDNA libraries several times, characterizing about 80 independent clones, and performed primer extension analysis primed at the position close to the 5’ end of HPC-113. Reverse transcription, however, failed to reach any further than HPC-113.

The deduced amino acid sequence had several distinct characters; they were an aspartate cluster of 7 residues, Asp54, including 1 serine, two possible N-linked glycosylation sites (Asn117-Arg118-Ser119, and Asn147-Ala148-Thr149), and some serine and threonine residues which were possible O-linked glycosylation sites in the sequence.

Computer searches of the two available protein sequence databases (see “Materials and Methods”) revealed that the HPC-1 antigen sequence, as a whole, showed no significant homology to known polypeptides, suggesting that the HPC-1 antigen is a protein of novel class. However, it should be noted that some local similarities with laminin, which is a major component of extracellular matrix (Refs. 38-40, Fig. 6) were found. Two partial sequences with such similarities are indicated by underlines in Fig. 6. Fragment a (Leu18-Val107) and fragment b (Ile54-Glu119) showed significant similarities to the partial sequences in the C-terminal domain I (40 kDa) of the mouse laminin B1 chain (41) (Fig. 8) at ratios of 39.4 and 33.3%. It was particularly remarkable that the serial 8 amino acid sequence in fragment b, NVEEVKRRK (No. 60-67), perfectly matched the corresponding sequence of the laminin B1 chain with only one conservative substitution of Val110 for Leu. Moreover, 35 residues in fragment b (Ala35-Glu119) were also similar (31.4%) to the C-terminal region of rat S-laminin, a variant of the laminin B1 chain localized specifically in neuromuscular junctions (42).

In the sequence of HPC-1 antigen, we unambiguously found heptad repeats (a-g) in Fig. 9, with hydrophobic acids at the a and d positions (Fig. 9). Although there are some exceptions of unfavorably charged or polar residues, these periodic sequences are characteristic of fibrous proteins folded as double or triple coiled-coil α-helices (43), such as myosin rod (44), nuclear lamin(s) (45), cytokeratin (46), virus hemagglutinin(s) (47,48), and the long arm of laminin B1 chains described above. Computerized secondary structure prediction analysis (30) also revealed three α-helical portions, which corresponded well to the heptad repeat regions (Fig. 9); we designated them H1, H2, and H3. The separation between H1 and

**DISCUSSION**

Cloning of cDNA for HPC-1 Antigen Molecule—In this study, we isolated rat cDNA clones for HPC-1 antigen(s) by expressing fusion proteins in bacterial cells. As the cDNA clones containing poly(A) tail were 2.2 kb, it is conceivable that these clones covered about 90% of the full-length mRNAs (2.4 kb) expected from the results of the RNA blotting (Fig. 4). However, we found no cDNA clone coding for a longer 5’ nucleotide sequence than HPC-113. The clones so far analyzed all terminated in almost the same 5’ area. A possible reason was that cDNA synthesis by reverse transcriptase stopped in this area presumably due to an inhibitory higher structure of mRNA. In fact, the computer analysis indicated the highest level of G-C content near the 5’ edge of cDNAs, which might form a complicated higher structure in mRNA.

**Tissue Expression of HPC-1 mRNA**—Despite the fact that RNA blot analysis detected HPC-1 mRNA only in neuronal
tissues (cerebral cortex, hippocampus, and cerebellum), RNA
PCR assay exhibited small amounts of HPC-1 gene transcript
in RNA and non-neuronal tissues. The non-neuronal HPC-
1 mRNA was not derived from any contamination of genomic
DNA, since the two primers used in this assay were in distinct
exons in rat HPC-1 chromosomal gene, and the size of the
PCR product was much smaller than the calculated ones.  
The non-neuronal transcript was slightly longer than the
neuronal one, although the signals were very weak (Fig. 5).
These results strongly suggested that the non-neuronal HPC-
1 transcript was closely related to but distinct from the
neuronal one. In the intestine, however, the size of the PCR
product was the same as that of the neuronal one. This signal
might originate in neurons of the intestinal plexuses, although
both the immunohistochemical and the in situ hybridization
studies revealed no HPC-1 signals in non-neuronal tissues.  
Considering both these results and PCR experiments, it was
concluded that HPC-1 was expressed predominantly in neu-
ronal tissues.

Topology and Heterogeneity of HPC-1 Antigen Molecule—
The deduced amino acid sequence of the HPC-1 antigen
includes a distinct hydrophobic sequence in the COOH-termi-
nal end (Fig. 6s and 7); thus HPC-1 antigen seemed to be
an integrated membrane protein. Although two other hydro-
phobic regions outside the prominent one were also observed,
using windows of 10 neighboring residues (Fig. 7), both of
them were considered inadequate for a typical membrane-
spanning region (28, 33).

The sequence analysis of the cDNA clone HPC-104 showed
that the HPC-1 epitope was limited in the region depicted in
Fig. 7 (bold bar in the lower sides) and mAb HPC-1 reacted
with living cultured cells. These results suggest that the
HPC-1 epitope and the portion of the NH2-terminal side from
the highly hydrophobic domain are on the extracellular do-
main. The HPC-1 antigen sequence does not seem to have an
NH2-terminal signal sequence including a possible signal se-
quence cleavage site (34, 35) nor a hydrophobic sequence near
the NH2-terminus, at least one of which is usually present in
a precursor of membrane bound proteins. Accordingly, it is
likely that the cDNA obtained so far does not cover the full-
length coding region of the HPC-1 antigen mRNA. Another
possibility, although unusual, is that Met11 is the authentic
translation initiation site, since the nucleotide sequence just
before Met11 is well matched to the consensus sequence for a
translation initiation site. In fact, we observed in vitro translation experiments with HPC-113 that the
translation was efficiently initiated at Met11 (Fig. 3, lanes f and g). In SDS-PAGE, the in vitro translation product exhibited
almost the same mobility to the lowest of the three bands
detected in the cerebral cortex (Fig. 3, lanes e–g), suggesting that this Met might be an initiation site. This question will
certainly need to be answered in the near future.

Characterization of the Structure of HPC-1 Antigen Mole-
cule—The deduced amino acid sequence of the HPC-1 antigen
as a whole revealed no strict homology with known polypep-
tides. Alignment of HPC-1 antigen sequence according to 28-
residue cycles represented periodic hydrophobic and nonpolar
residues (Fig. 9). These periodic sequences are characteristic
of proteins forming coiled-coil structures, suggesting the pos-
sibility that the HPC-1 antigen molecule exists as dimer or
tramer in the physiological states.
The local homology to laminin B1 chain is of particular
interest. Laminin can interact with other extracellular matrix
components and possesses multiple biological functions such as
stimulation of growth and differentiation of cells, neurite
outgrowth promotion, and cell attachment and migration (38,
39, 49). Fragments a and b in the HPC-1 antigen were homologous to the two distinct partial sequences in laminin B1
chain, both of which were involved in the region with neurite
growth-promoting activity (50, 51) (Fig. 8). It has been
reported that the biologically functional regions in laminin A
and B2 chains are confined to the relatively short peptides
(52-54), but there has been little information regarding the
structure-function relationship of the B1 chain. Accordingly,
it is difficult to assess the meanings of these homologies.
The seriously matched sequences in fragment b, NVEEVKRR,
however, did imply some functional correlation between HPC-
1 antigen and laminin. We also note the similarity between
HPC-1 antigen and s-laminin which is an adhesive molecule
involved in the targeting of motor neurons onto synaptic
clefts (56). Therefore, it is possible that the HPC-1 antigen
might participate in such cell-cell interactions between neu-
rons, although at present the biological function of the HPC-
1 antigen is not known, and further studies are necessary. In
this context, the cDNA probe and the antiserum reported in
this study will be very useful tools for studying biological
functions and tissue distribution of the HPC-1 antigen or its
gene expression.

Acknowledgments—We thank Drs. Tomoki Shirao, Nobuhiko Ko-
jima, Kiyokazu Agata, Akira Nagafuchi, Takashi Nakayama, Sonoko
Furuya, and Hideto Kuwayama for their helpful and instructive
discussions in this study, Dr. Kazuhiro Amanai for his help in the
synthesis of oligonucleotides, and many colleagues of Okazaki
Research Institutes for their technical support. We are very grateful to
Professor Setsuro Ebashi for his continuing encouragement.

REFERENCES
785–803
847–855
J. Neurosci. 4, 2025–2042
Sci. U. S. A. 81, 6255–6259
Brain Res. 20, 266–289
26, 133–143
11. Akagawa, K., and Barnstable, C. J. (1986) Brain Res. 383,
110–120
12. Akagawa, K., and Barnstable, C. J. (1987) Brain Res. 408,
154–163
Brain Res. 518, 1–5
437, 295–308
31, 124–128
cular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring
Harbor Laboratory Press, Cold Spring Harbor, NY
U. S. A. 80, 1194–1198
cloning (Glover, D. M., ed) Vol. 1, pp. 49–78, Academic Press,
New York
137, 286–287

2 A. Inoue, K. Obata, and K. Akagawa, unpublished data.
cDNA Cloning of HPC-1 Antigen

31. Deleted in proof
55. Deleted in proof