weeks, numbers were highest when Dulbecco's modified Eagle's medium (DMEM) was supplemented with 5% adult human serum, but long-term survival was better with 5% fetal bovine serum (FBS). We tested the effects of interleukin-3 and GM-CSF (AMGEN) and of highly purified recombinant human M-CSF, long and short clones [M. B. Ladner *et al.*, *EMBO J.* **6**, 2693 (1987); a gift of Cetus Corp., Emeryville, CA], all factors known to enhance the growth and survival of cells of the monocyte-macrophage lineage or microglial cells (D. Giulian and J. E. Ingeman, *J. Neurosci.* **8**, 4707 (1988)]. Addition of 500 to 1000 units of growth factor per milliliter of defined medium [P. A. Eccleston and D. H. Silberberg, *Dev. Brain Res.* **16**, 1 (1984)] or medium with 5% FBS was compared to addition of 10% GCT. None of these factors or GCT induced microglial cell division, since LDL-R^+ cells were not labeled by tritiated thymidine after a 24-hour labeling period, as determined by whole-cell autoradiography. Numbers of surviving microglial cells after 2 weeks were highest in medium containing 10% GCT and 5% FBS compared with all other factors tested either singly or in combinations of two.

19. Viral strains. (i) HIV-$1_{AD87(M)}$: HIV-1_{Ada} passaged 12 times in macrophages (*2*); stock was grown in PBLs. RT level of stock was 7×10^5 cpm/ml. (ii) HIV-1_{BaL}: Routinely passaged only in macrophages. A gift from S. Gartner in R. Gallo's laboratory, National Cancer Institute (NCI). RT level of stock was 8×10^5 cpm/ml. (iii) HIV-$1_{HTLV\text{-}IIIB}$: Original isolate [M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *Science* **224**, 506 (1984)], passaged in PBLs and H9 T cell line. RT level of stock was 8.0×10^5 cpm/ml. Infectivity was checked in H9 T cell line. (iv) HIV-1_{LAV}: Original isolate [F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983)], passaged in PBLs and A-301 T cell line. RT level of stock was 1.5×10^6 cpm/ml. Infectivity checked in HeLa T4 cells, H9 T cell line, and PBLs. (v) HIV-1_{A1638}: Primary isolate from PBLs of an asymptomatic seropositive patient passaged once in macrophages or PBLs. A gift from A. Valentin and B. Asjo of Karolinska Institute, Stockholm. RT level of stock was 1.5×10^4 cpm/ml. (vi) HIV-2_{B59}: Primary isolate from PBL of an asymptomatic patient passaged once in macrophages or PBLs. A gift from A. Valentin and B. Asjo of Karolinska Institute, Stockholm. RT levels of stocks were 1×10^5 to 2×10^5 cpm/ml. (vii) HIV-$2_{sbl/isy}$: Molecularly cloned virus grown in Hut 78 cells; a gift from G. Franchini, NCI [G. Franchini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2437 (1989); N. Hattori *et al.*, *ibid.*, in press].
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21. HIV-1 p24 assay kit was purchased from Du Pont Biotechnology Systems and used as described [Y. Koyanagi *et al.*, *Science* **236**, 819 (1987)]. Expression of *gag* antigens in cultures infected with HIV-2 was assessed by indirect immunofluorescence with antibodies for simian immunodeficiency virus (SIV-1) p24 cross-reacting with HIV-2 p24 (a gift from G. Franchini, NCI). Generally, no immunofluorescence staining was seen in HIV-2–infected cultures; however, some staining was seen in cytoplasmic inclusions of LDL^+ cells in one set of cultures infected with HIV-$2_{sbl/isy}$, but this was not accompanied by an increase in supernatant RT levels.
22. Estimated peak HIV production per microglial cell: Peak RT activity production in microglial cells infected with HIV-$1_{AD87(M)}$ varies from 16 to 54 cpm per cell in different experiments. When infected under similar conditions with HIV-$1_{AD87(M)}$, blood-derived monocyte-macrophages produced ~36 cpm RT activity per cell. Lower production of RT activity per cell (7 cpm) was observed in PBL cultures infected with HIV-$1_{AD87(M)}$, whereas HIV-1_{LAV} infection of these cells resulted in the production of ~41 cpm of RT activity per cell. The level of HIV production by microglial cells is similar to the levels produced by macrophages infected with similar strains in our experiments, as well as those in previous publications (*1*, *2*).
23. Anti–HIV-1 p17 antibody was a gift from F. Veronese, NCI, and Bionetics Research, Inc., Rockville, MD [F. D. Veronese, T. D. Copeland, S. Oroszlan, R. C. Gallo, M. G. Sarngadharan, *J. Virol.* **62**, 795 (1988)].
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Protein Tyrosine Phosphatase Activity of an Essential Virulence Determinant in *Yersinia*

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***Yersinia* is the genus of bacteria that is the causative agent in plague or the black death, and on several occasions this organism has killed a significant portion of the world's population. An essential virulence determinant of *Yersinia* was shown to be a protein tyrosine phosphatase. The recombinant 50-kilodalton *Yersinia* phosphatase had a specificity for removal of phosphate from Tyr-containing as opposed to Ser/Thr-containing phosphopeptides and proteins. Site-directed mutagenesis was used to show that the *Yersinia* phosphatase possesses an essential Cys residue required for catalysis. Amino acids surrounding an essential Cys residue are highly conserved, as are other amino acids in the *Yersinia* and mammalian protein tyrosine phosphatases, suggesting that they use a common catalytic mechanism.**

PROTEIN TYROSINE PHOSPHORYLation is an early event in the signal transduction pathway used by several receptors involved in mediating cellular proliferation and regulation. Tyrosine phosphorylation can also lead to unrestrained cell growth, and a number of tyrosine kinases appear to function as oncogenes (*1*). Tyrosine phosphorylation has been shown to be a key factor in the regulation of the cell cycle (*2*). The levels of protein tyrosine phosphorylation appear to be modulated within the cell by both tyrosine kinases and protein tyrosine phosphatases (PTPases) (*3*). Evidence indicates that PTPases constitute a family of enzymes that most likely function in regulating the extent and duration of tyrosine phosphorylation within the cell (*3*). Tonks *et al.* (*4*) reported the purification of a 35-kD protein tyrosine phosphatase (PTPase 1B) from human placenta, and Charbonneau *et al.* (*5*) showed that this phosphatase shared significant amino acid sequence homology with the cytoplasmic domain of the leukocyte cell surface glycoprotein, CD45. CD45 was subsequently shown to have tyrosine phosphatase activity (*6*). Streuli *et al.* (*7*) have cloned another member of the family called LAR (leukocyte antigen–related protein), which is a tyrosine phosphatase structurally related to CD45.

Work from our laboratory suggests that there are additional members of this family of PTPases that resemble CD45 and LAR (*8*). In our initial screening of a rat brain cDNA library, we isolated several PTPase clones. We have found the cloning, expression, and localization of a PTPase cDNA from rat brain that encodes a protein having 97% identity with the protein sequence reported by Charbonneau *et al.* (*8*, *9*). We have referred to the protein as PTP 1. Cool *et al.* (*10*) have cloned a structurally related PTPase from a human T cell cDNA library. Another clone from our cDNA library has been designated as PTP 18 (*11*). The longest PTP 18 cDNA, which is approximately 5.6 kb in length, encodes an enzyme having an

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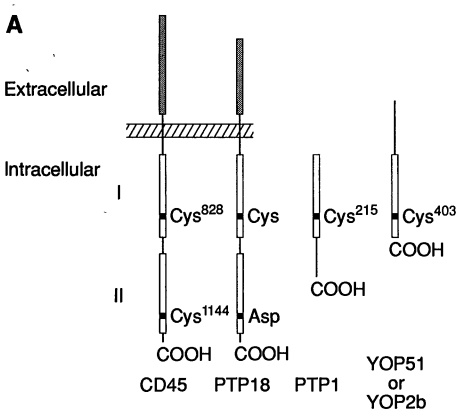
external domain, a transmembrane region, and two repeated PTPase domains (Fig. 1A). Collectively, the PTPases appear to fall into two "families." One subgroup includes those having an external domain as well as two duplicated cytoplasmic PTPase domains. Members of this subgroup include CD45 and LAR. The other subgroup includes PTP 1 and the T cell PTPase, both of which have a single catalytic domain and no extracellular region. Streuli *et al.* (*12*) have shown that the tyrosine phosphatase activity of CD45 resides solely in PTPase domain 1. When Cys^{828} of CD45 was mutated to Ser, no tyrosine phosphatase activity was observed. We have made similar observations using site-directed mutants of PTP 1, where Cys^{215} was changed to either Ala or Ser (*13*). In both cases, no tyrosine phosphatase activity was observed. We have also prepared a number of COOH-terminal deletions of PTP 1, and analysis of these mutants indicates that residues between 7 and 284 are required for catalysis (*13*).

To further define the amino acid residues important in catalysis, we aligned PTP 1 and domain 1 of LAR, CD45, PTP 18, dLAR, and dPTP (*12*). Using the boundaries defined by our deletion analysis of PTP 1, we searched the National Biomedical Research Foundation (NBRF) database (release 21.0-6/89) with the FASTA program (*14*) for additional proteins that share sequence identity with the catalytic domains of the PTPases (Fig. 1B). The PTPase family dominated our similarity search. However, one protein also showed a pattern of invariant residues highly characteristic of the PTPase family. This protein, YOP 2b (*15*), has the highest degree of identity with the first catalytic domain of PTP 18 and had a Z value of 10.1 [Z values (observed score − mean of shuffled score)/(SD of shuffled scores) of 10 or more are considered to be indicative of probable similarity between two proteins (*14*).] The YOP 2b protein is encoded on a 70-kb plasmid present in the bacterium *Yersinia pseudotuberculosis* (*15*).

The genus *Yersinia* is comprised of three species of bacteria that are causative agents in human disease. *Yersinia pestis* is the pathogen responsible for the plague, also known as the black death because it reduced the population of Europe by some 25 million in the 15th century (*16*). *Yersinia pseudotuberculosis* causes diarrhea, emaciation, and death, whereas *Y. enterocolitica* results in a range of gastrointestinal syndromes (*17*). Virulence of all three *Yersinia* species is associated with plasmid-encoded proteins (*17*). The YOP H gene, which encodes the YOP 2b protein, has been shown to be obligatory for pathogenesis (*15*). Plasmids that have a nonfunctional YOP H gene are avirulent (*15*). *Yersinia enterocolitica* contains a similar protein, YOP 51, which shows 99% amino acid identity to YOP 2b (*18*).

This degree of identity between the two proteins suggests that they have common key roles in pathogenesis and disease. The YOP proteins are highly conserved in all three *Yersinia* species and appear to be related immunologically and by protein mass (*19*). However, the sequences of the *Y. pestis* equivalent of YOP 2b or YOP 51 has not been reported (*19*). The alignment of the invariant residues of YOP 2b or YOP 51 with known PTPases suggests that these bacterial proteins might possess protein tyrosine phosphatase activity (Fig. 1B). The virulence of these proteins could be based on their ability to dephosphorylate phosphotyrosine-containing proteins that participate in the regulation of mammalian cell function.

To demonstrate that the YOP 51 protein is indeed a tyrosine phosphatase, we ampli-



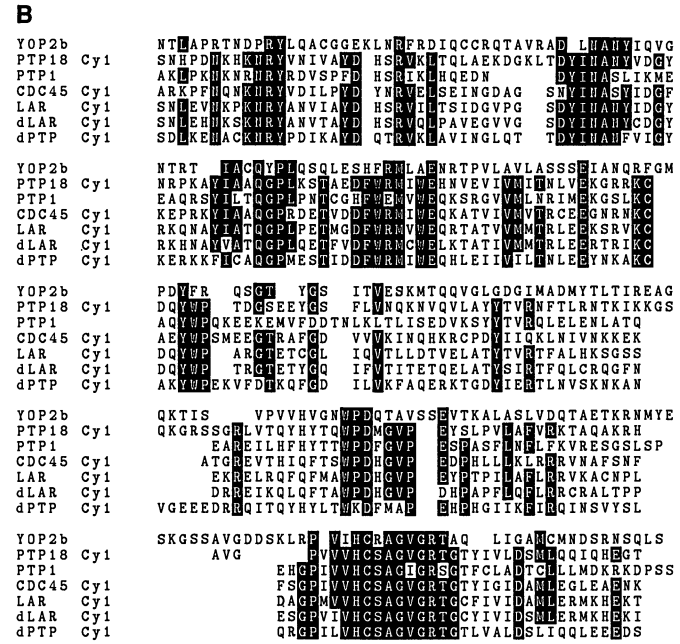


Fig. 1. Comparison of PTPase family. (**A**) Schematic alignment of PTPases. Open and stippled segments represent the PTPase and extracellular domains, respectively. the solid black segments denote the conserved sequences surrounding the essential Cys residue. (**B**) Sequence alignment of the PTPase domain of YOP 2b (*15*), PTP 1 (*8*), and the PTPase domain I of PTP 18, CD45 (*12*), LAR (*7*), dLAR (*12*), and dPTP (*12*). Cy 1 refers to the cytosolic PTPase domain I. Residues in the black regions indicate positions where five or more of the seven sequences are identical. Gaps are also introduced as blank space. For orientation purposes, PTP 1 starts at residue 34 and ends at residue 285. Residues 206 to 468 of YOP 2b are shown in the figure. The essential Cys residue is Cys^{215} in PTP 1 and Cys^{403} in YOP 2b. Arg^{211} and Gly^{397} in YOP 2b are substituted in YOP 51 by Ala^{211} and Ala^{397}, respectively.

Table 1. Substrate specificity of YOP 51. Tyrosine phosphorylation was catalyzed by a recombinant $p43^{v\text{-}abl}$ tyrosine kinase. Phosphorylation on Ser and Thr was achieved with the catalytic subunit of cAMP-dependent protein kinase. PTPase activity was measured at a phosphorylated substrate concentration of 5 nM. A level equal to approximately 0.1% of the tyrosine phosphate hydrolysis of Raytide could be detected in this assay. ND, not detectable. The specific activities of YOP 51 were determined at three enzyme concentrations, enough to reach approximately 30, 50, and 70% dephosphorylation of substrates. Data were obtained by triplicated assays.

Substrate	Phosphorylated amino acids*	Phosphatase activity ± SEM (pmol/min/mg of protein)
Angiotensin	Tyr	0.32 ±0.02
Raytide*	Tyr	0.8 ± 0.02
RR-Src†	Tyr	0.08 ± 0.008
Casein	Ser/Thr	ND
Histone H2a	Ser/Thr	ND

*Gastrin analog (Oncogene Science, Manhasset, NY). †The amino acid sequence of the peptide is Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly.

fied the corresponding gene using the polymerase chain reaction from plasmid pTM200, which contains the complete coding sequence of YOP 51 (*20*). The amplified DNA (1.4 kb) was inserted into the *Escherichia coli* expression vector pT7-7, which uses the bacteriophage T7 promoter (*21*). The resulting construct, T7–YOP 51, was used to express the YOP 51 protein with methods essentially as previously described (*9*). Extracts were prepared from bacteria containing the pT7–YOP 51 or the pT7-7 plasmid and examined for protein tyrosine phosphatase activity (*9, 22*). Dephosphorylation of a tyrosine phosphate–containing peptide increased with the addition of increasing amounts of the extract from bacteria harboring the pT7–YOP 51 plasmid, but not with control extracts (Fig. 2A). The dephosphorylation reaction was dependent on both the incubation time and enzyme concentration.

The substrate specificity of the recombinant YOP 51 activity was examined with either ^{32}P-labeled Tyr-containing or Ser/Thr-containing phosphopeptides or proteins (*22*); the recombinant YOP 51 protein was specific for phosphotyrosine (Table 1). Differences in the rate of tyrosine dephosphorylation were also observed with the three substrates examined, although these measurements were made under nonsaturating substrate concentrations. Casein or histone H2a phosphorylated on Ser/Thr were not substrates for the recombinant YOP 51 protein while they were dephosphorylated by acid phosphatases.

To determine whether the recombinant YOP 51 protein utilizes residues similar to those used by the mammalian PTPases in catalyzing tyrosine phosphate hydrolysis, we used site-directed mutagenesis to change Cys^{403} of YOP 51 to either Ala or Ser (*23*). Substitution of either amino acid at this position caused a loss of enzymatic activity (Fig. 2B). Expression of the recombinant and mutant YOP 51 proteins was verified by incubation of plasmid-containing bacteria with ^{35}S-labeled Met in the presence of rifampicin. Rifampicin selectively blocks bacterial RNA polymerase, but leaves T7-polymerase unaffected (*21*). All three plasmids expressed radiolabeled proteins of approximately 50 kD as assessed by SDS–polyacrylamide gel electrophoresis. In addition, a plasmid encoding residues 154 to 468 of YOP 51 (pT7-YOP-PTP), which corresponds to the PTPase catalytic domain, also possessed tyrosine phosphatase activity. We have also shown that the tyrosine phosphorylated rat insulin receptor is rapidly dephosphorylated by the recombinant YOP protein, but not with the YOP protein where Cys^{403} was mutated to Ser. Collectively, these results show that the YOP 51 protein and most likely, the corresponding proteins produced by *Y. pestis* and *Y. pseudotuberculosis*, are all tyrosine phosphatases that need an essential Cys residue for catalysis. The presence of highly conserved amino acids surrounding this essential catalytic residue, as well as amino acids in other regions of the protein (Fig. 1B), implies a common catalytic mechanism for the YOP 51 protein and the mammalian protein tyrosine phosphatases. It will be necessary to examine the substrate preference of the recombinant YOP 51 protein with additional physiologically relevant substrates such as the epidermal growth factor (EGF) receptor and cdc2. In addition, purification of the recombinant YOP 51 protein will provide a measure of its relative phosphatase activity and allow us to compare this value with PTP 1 or CD45.

Our results raise a number of questions. Although the mechanism of pathogenesis is not understood, the fact that the YOP proteins are found in the bacterial outer membrane or are released in the culture medium is of particular interest (*19*). It is possible that after infection, the bacterial YOP proteins are secreted into the host cell, which leads to the dephosphorylation of proteins important in the signal transduction pathway or cell cycle regulation in mammalian cells (*2*). Also, since bacteria do not contain tyrosine phosphate, one must address the question concerning the origin of the plasmid-encoded *Yersinia* PTPase. One cannot help but be reminded that retroviral-encoded tyrosine kinases are thought to have arisen from the hosts' genome (*1*). A similar mechanism may be responsible for derivation of the plasmid-encoded PTPases harbored by certain pathogenic bacteria.

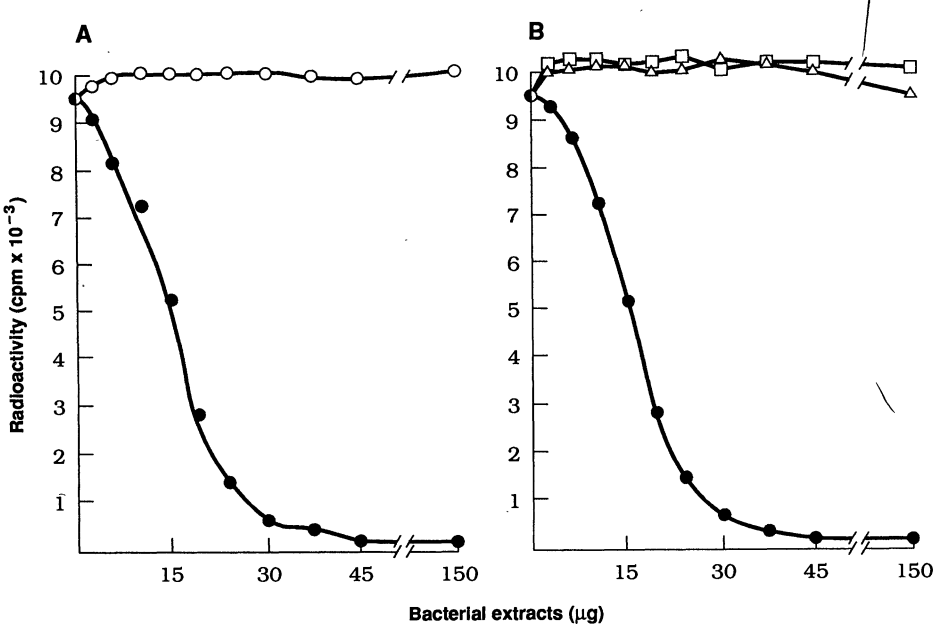


Fig. 2. Expression and activity determination of recombinant YOP 51. (**A**) Dephosphorylation of Raytide by recombinant YOP 51. *Y*-axis represents the radioactivity remaining on phosphocellulose paper. Extracts from bacteria harboring pT7-7 and pT7–YOP 51 are indicated by open (○) and closed (●) circles, respectively. (**B**) Dephosphorylation of Raytide with recombinant YOP 51 (●) and the site-directed mutants; C403A (□) and C403S (△).

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11. Clone PTP 18 was isolated by screening the rat brain cDNA library with a degenerative oligonucleotide (*8*). The cDNA fragments were subcloned into M13 and sequenced by the dideoxy chain termination method. Standard molecular biology techniques were used [T. Maniatis, E. H. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986)]. The sequence data has been submitted to European Molecular Biology Laboratory/GenBank Data Library.
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22. Phosphorylation of tyrosine in angiotensin, RR-Src peptide, and Raytide was catalyzed with p43$^{v\text{-}abl}$ protein tyrosine kinase following manufacturer's recommendation (Oncogene Science). Phosphorylated peptide was separated from unreacted ^{32}P-labeled adenosine triphosphate (ATP) by ion exchange on phosphocellulose paper and elution with 0.5 M $(NH_4)HCO_3$ after extensive washing. Phosphorylation on Ser and Thr of histone H2a and casein was achieved with the catalytic subunit of cAMP-dependent protein kinase. Dephosphorylation was routinely performed in 20 μl of imidazole buffer, pH 7.5, containing 5 nM of phosphorylated substrate at an incubation temperature of 25°C for 10 min.
23. The YOP 51 coding sequence was subcloned from pT7–YOP 51 into M13MP18 for site-directed mutagenesis. Cys^{403} was mutated to either Ala or Ser with the in vitro mutagenesis kit (Amersham). The mutated DNA was subcloned into pT7-7 for expression.
24. Supported by grants from the NIH (National Institute of Diabetes and Digestive and Kidney Diseases 18849) and the Walther Cancer Institute. We would like to thank G. R. Cornelis, Université Catholique de Louvain, Brussels, Belgium, for plasmid pTM 200; J. D. Corbin, Vanderbilt University, School of Medicine, for the catalytic subunit of protein kinase A; K.-H. Kim (Purdue University) for the purified rat insulin receptor; H. Qiu for technical assistance; and the Purdue AIDS Center Grant (A127713) for support of the computer facilities. Special thanks to T. Woodford, D. Pot, and E. Remboutsika for helpful discussions and reading the manuscript. This is journal paper number 12486 from the Purdue University Agricultural Experimentation Station.

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Table 1. Agonist-evoked current amplitudes of glutamate receptors expressed in vitro. Numbers represent average amplitudes (mean ± SEM) of inward currents (pA) evoked by agonist at a holding potential of −60 mV. Values of *n* are in parentheses below.

Receptors	Agonists (100 μM)	
	L-Glu	Kainate
GluR-A	32 ± 9 (9)	46 ± 13 (8)
GluR-B	8 ± 6 (4)	25 ± 12 (7)
GluR-C	7 ± 3 (6)	30 ± 7 (6)
GluR-D	22 ± 6 (6)	41 ± 11 (6)
GluR-A, -B	68 ± 16 (15)	216 ± 32 (17)
GluR-B, -D	13 ± 3 (4)	160 ± 25 (4)
GluR-A, -B, -C, -D	16 ± 17 (6)	107 ± 32 (6)

A Family of AMPA-Selective Glutamate Receptors

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Four cloned cDNAs encoding 900–amino acid putative glutamate receptors with approximately 70 percent sequence identity were isolated from a rat brain cDNA library. In situ hybridization revealed differential expression patterns of the cognate mRNAs throughout the brain. Functional expression of the cDNAs in cultured mammalian cells generated receptors displaying α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)–selective binding pharmacology (AMPA = quisqualate>glutamate>kainate) as well as cation channels gated by glutamate, AMPA, and kainate and blocked by 6,7-dinitroquinoxaline-2,3-dione (CNQX).

MOST NEURONS IN THE CENTRAL nervous system are excited by glutamate. The postsynaptic actions of this neurotransmitter are mediated by at least three major, pharmacologically distinct classes of ionotropic receptors as well as by a metabotropic receptor at which glutamate modulates the release of intracellular Ca^{2+} (*1*). The ionotropic receptors contain glutamate-gated cation channels and are named according to their selective agonists as the NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), and kainate receptors. The pharmacological and electrophysiological properties of NMDA receptors are well studied (*1*). However, because there are no selective antagonists, the characterization of the non-NMDA receptors is less advanced, and it has been difficult to distinguish kainate and AMPA receptors (*2*). Non-NMDA receptors are the major mediators of fast glutamatergic neurotransmission and may contribute to synaptic plasticity (*3*). Glutamate-induced neurotoxicity, a pathological process of widespread clinical importance also involves these receptors (*4*). Hence, the key role played by glutamate-operated channels in virtually all physiological and many abnormal brain functions accounts for the ever increasing efforts to elucidate their molecular design.

Recently, Hollman and co-workers characterized a molecule designated GluR-Kl (*5*), the sequence of which was similar to kainate binding proteins of chick and frog (*6*). To study the diversity of this family and the pharmacology of its members, we isolated cDNAs encoding several sequence-related novel receptors. We obtained a result that was unexpected from the assignment of GluR-Kl as a kainate receptor subtype (*5*): these polypeptides made up a family of AMPA receptors as indicated by their functional expression and distribution of their mRNAs in rat brain.

Cloned cDNAs encoding the receptors were obtained via polymerase chain reaction (PCR)–mediated DNA amplification by using published sequences (*5, 6*) for primer design and by the subsequent screening of cDNA libraries constructed from rat brain mRNA. Four molecular species, designated GluR-A to -D, were fully characterized with GluR-A showing 100% sequence identity to GluR-Kl. The four predicted polypeptide sequences (Fig. 1), each approximately 900 amino acids in length including a signal sequence, revealed upon pairwise comparisons overall similarities between 70% (GluR-A versus -B) and 73% (GluR-B versus -C). Similarities dropped to between 56% (GluR-A versus -D) and 63% (GluR-C versus -D) when comparison was restricted to the NH_2-terminal 470 amino acids of the mature polypeptides. This region contained the highest number of substitutions in the four receptors but specified the only con-

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