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Hippocampal Plasticity Involves Extensive Gene Induction and Multiple Cellular Mechanisms**

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Abstract

Long-term plasticity of the central nervous system (CNS) involves induction of a set of genes whose identity is incompletely characterized. To identify candidate plasticity-related genes (CPGs), we conducted an exhaustive screen for genes that undergo induction or downregulation in the hippocampus dentate gyrus (DG) following animal treatment with the potent glutamate analog, kainate. The screen yielded 362 upregulated CPGs and 41 downregulated transcripts (dCPGs). Of these, 66 CPGs and 5 dCPGs are known genes that encode for a variety of signal transduction proteins, transcription factors, and structural proteins. Seven novel CPGs predict the following putative functions: *cpg2*—a dystrophin-like cytoskeletal protein; *cpg4* —a heat-shock protein: *cpg16*—a protein kinase; *cpg20*—a transcription factor; *cpg21*—a dual-specificity MAP-kinase phosphatase; and *cpg30* and *cpg38*—two new seven-transmembrane domain receptors. Experiments performed in vitro and with cultured hippocampal cells confirmed the ability of the *cpg-21* product to inactivate the MAP-kinase. To test relevance to neural plasticity, 66 CPGs were tested for induction by stimuli producing long-term potentiation (LTP). Approxi-

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**This article is dedicated to the memory of our mentor, friend, and colleague, Dr. Yoav Citri, who died tragically in a road accident on December 13, 1995.

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mately one-fourth of the genes examined were upregulated by LTP. These results indicate that an extensive genetic response is induced in mammalian brain after glutamate receptor activation, and imply that a significant proportion of this activity is coinduced by LTP. Based on the identified CPGs, it is conceivable that multiple cellular mechanisms underlie long-term plasticity of the nervous system.

Index Entries: Dentate gyrus; gene expression, glutamate; hippocampus; neural plasticity; synapse; signal transduction.

Introduction

Plasticity is a property of the brain that underlies learning and memory processes, and is also involved in the refinement of neuronal connections during nervous system development (Shatz, 1990; Kandel and O'Dell, 1992; Bliss and Collinridge, 1993). Plasticity changes occur at the synapse, where they are expressed as activity-dependent changes in the efficacy of synaptic transmission. Many plasticity-related changes in the mammalian central nervous system (CNS) are induced by activation of excitatory glutamate receptors. *De novo* activation of gene transcription participates in long-term plasticity, and glutamate is likely to cause induction of the relevant genes (Goelet et al., 1986; Sheng and Greenberg, 1990; Huang et al., 1994; Nguyen et al., 1994). The cyclic-AMP-(cAMP) activated transcription factor, CREB, has been implicated in memory-related plasticity changes (Burtchuladze et al., 1994). CREB and the products of many immediate-early genes (e.g., c-*fos* and *zif268*) induce expression of downstream genes whose products are likely to produce synaptic changes (Frank and Greenberg, 1994). The nature of these downstream candidate plasticity-related genes (CPGs) may illuminate the types of processes responsible for long-term plasticity changes.

The dentate gyrus (DG) of the hippocampus is an appropriate source for cloning plasticity-related genes, because it exhibits marked plasticity following electrophysiological or pharmacological stimulation (Ben-Ari and Represa, 1990; Bliss and Collinridge, 1993). Activation of DG neurons by the potent glutamate analog, kainate, results in prominent, plasticity-related, physiological and morphological changes (Tauck and Nadler, 1985; Ben-Ari and Represa, 1990; Sloviter, 1992). Importantly, the granular neurons of the DG are not susceptible to excitotoxic damage induced by kainate activation (Ben-Ari, 1985; Smeyene et al., 1993). Many studies have examined the involvement of specific genes, primarily immediate-early genes (IEGs) (Cole et al., 1989; Sheng and Greenberg, 1990; Morgan and Curran, 1991), and genes encoding neurotrophic factors (Gall, 1988) in hippocampal plasticity. Other studies used differential cDNA cloning to isolate genes involved in plasticity (Qian et al., 1993; Yamagata et al., 1994a,b; Lyford et al., 1995).

To obtain an unbiased and comprehensive view of the population of activity-modulated genes, we employed a highly sensitive differential cDNA cloning protocol. Initially, 1000 cDNA clones of a kainate-activated, subtracted DG cDNA library were screened, and 52 kainate-induced CPG cDNAs cloned (Nedivi et al., 1993). Here we present a 10-fold larger screen. Individual screening of approx 10,000 subtracted cDNA clones, by using a "clone-by-clone" strategy, resulted in the identification of 362 different CPGs. By contrast, 41 transcripts whose transcription is apparently downregulated by glutamate were identified, implying that extensive gene induction, rather than repression, plays a dominant role in affecting neural plasticity. The identity of the cloned CPGs and dCPGs appears to reflect involvement of multiple biochemical mechanisms in the response to glutamate.

Experimental Procedures

The "Clone-by-Clone" Screening Strategy

The "clone-by-clone" screen for CPGs comprised the following steps:

1. Constructing a subtracted library from poly(A)$^+$ RNA of kainate-activated DG as we previously described (Nedivi et al., 1993). Briefly, ubiquitous activated DG cDNA sequences were subtracted by hybridization to excess poly(A)$^+$ RNA derived from whole rat brain.
2. Picking phages (in λ-ZAP) from the unamplified subtracted library and converting them individually into plasmids (pBluescript). Plasmid DNA was digested to release the cDNA insert, separated on duplicate agarose gels, and transferred into nitrocellulose filters (*see* Table 1; 10,015 library clones were thus processed).
3. Primary screen: hybridizing the duplicate blots to high concentrations of cDNA probes. One probe was prepared from poly(A)$^+$ RNA of kainate-activated DG, and the second from nonactivated DG.
4. Identifying clones that exhibited more pronounced reaction with the kainate-activated probe, as compared to the control, nonactivated, cDNA probe (Table 1: positives on 1° screen). In a similar fashion, clones exhibiting a reduced hybridization signal with the kainate-activated probe (downregulated CPGs—dCPGs) were also selected.
5. Secondary screen: repeating the same type of Southern screen (steps 2–4) for all positive clones identified in the primary screen (Table 1: positives on 2° screen). The probes of the secondary screen were synthesized independently from different RNA preparations than those used in the primary screen.
6. Clones positive in the primary and secondary screens were subjected to partial nucleotide sequence analysis. Sequence comparisons to available DNA databases, including our database of previously cloned CPGs (Nedivi et al., 1993), were used to determine whether a differentially expressed cDNA was new or an already known sequence.

Plasmid Rescue from Individual Library Phages

Individual plaques were picked into 1 mL of 1xλdil buffer (10 m*M* Tris-HCl, pH 7.5, 10 m*M*, $MgSO_4$) containing 50 µL chloroform and stored at 4°C. Two hundred microliters of phage supernatant was used to infect 200 mL of overnight culture of *Escherichia coli* XL1-blue cells, in the presence of 1 µL ExAssist helper phage (Stratagene, La Jolla, CA). After 15 min at 37°C, 0.3 mL LB solution was added and samples were mixed vigorously at 37°C for 3 h. One and a half milliliters were heat-inactivated (20 min at 70°C) and centrifuged (8000 rpm in an Eppendorf microfuge). Supernatants were transferred into fresh tubes (stored at 4°C) and used (10 µL) to infect *E. coli* SOLR (Stratagene) bacterial cells (100 µL) for 15 min at 37°C. Bacteria (20–30 µL) were plated on LB agar plates containing ampicillin and incubated overnight at 37°C. Single colonies were then incubated for 7 h at 37°C. An aliquot (3 mL) of each culture was pelleted (1 min at 9000*g*), and DNA prepared according to the alkaline lysis method (Sambrook et al., 1989).

cDNA Synthesis

Poly(A)$^+$ RNA from DG of kainate-activated or from control rats was treated with RQ-1 DNase I (Promega, Madison, WI), followed by proteinase K (Sigma, St. Louis, MO). The RNA quality was tested by visualization on agarose gel, determination of absorbance (260/280 nm), and by Northern blot analyses (probed for induction of proenkephalin and *cpg1* and normalized to glyceraldehyde-3-phosphate-dehydrogenase RNA levels [Nedivi et al., 1993]). For cDNA synthesis, 5 µg poly(A)$^+$ RNA were incubated in 19 µL water with 2.5 µg oligo-dT (15-mer) primer (Promega) at 70°C for 10 min, followed by cooling on ice. Reverse transcriptase (SuperscriptII, 200 U; BRL, Bethesda, MD) was added for 30 min at 45°C, in a final reaction volume of 50 µL containing H-RT buffer (BRL), 30 U RNasin (Promega), the four deoxynucleotides (0.5 m*M* each), 10 m*M* DTT, and 30 µCi [α-^{32}P]dCTP (Amersham, Arlington Heights, IL). A second aliquot of SuperscriptII (200 U) was added and the incubation continued for 30 additional minutes. The reaction was stopped on ice with 10 µL NaOH (1 *N*) and 2 µL EDTA (0.5 *M*). The volume was then adjusted to 100 µL, and the mixture incubated for 20 min at 68°C for RNA hydrolysis. Ten microliters of HCl 1 *N* and 2 U yeast tRNA (Sigma) were added. A sample (1 µL) was used for radioactive counting, and the rest of the cDNA was purified on a Sephadex G-50 spin column. The eluate was sampled (1 µL for radioactive counting, and the amount of cDNA synthesized calculated. For probe labeling, 75 ng cDNA were incubated in 15 µL water with random hexamer primers (0.5 U, Boehringer; 5 min at 68°C)

Table 1
Summary of the "Clone-by-Clone" Screen of the Subtracted Kainate-Activated DG cDNA Library for CPGs

		% of total clones screened	% of recombinants
Total clones screened	10,015	100	
Clones with cDNA insert (recombinants)	7500	74.9	100
Recombinants producing a signal	5938	59.3	79.2
Positive clones on 1° screen	1243	12.4	16.6
Positive clones on 2° screen	655	6.5	8.7
Independent CPGs	362	3.6	4.8
Known genes	66	0.7	0.9
New genes	292	2.9	3.9
Independent dCPGs	41	0.4	0.5
Known genes	5	0.05	0.06
New genes	36	0.4	0.5

and put on ice. The cDNA was then added to a reaction mix (300 µL, final volume) containing 10 m*M* DTT, 90 m*M* HEPES buffer, pH 6.6, 10 m*M* $MgCl_2$, deoxynucleotides (dATP, dGTP, and dTTP) at 0.5 m*M* each, 1 µCi [α-^{32}P]dCTP (Amersham, 3000 Ci/mmol), and 100 U Klenow DNA polymerase (Amersham). The reaction was incubated overnight at room temperature, 200 µg herring sperm carrier DNA were then added, and the mixture spun through a Sephadex G-50 column.

DNA Hybridization

The Southern blot filters were prehybridized overnight at 42°C in 50% formamide, 5X SSCPE (0.6 *M* NaCl, 75 m*M* Na-citrate, 65 m*M* KH_2PO_4, and 5 m*M* EDTA, pH 7.2), 0.5% SDS, 5X Denhardt's solution (Sambrook et al., 1989), and 0.5 mg/mL herring sperm DNA. The prehybridization solution was then replaced by a fresh solution containing labeled cDNA probes at 50–100 × 10^6 cpm/ml. The radioactivity of kainate-activated DG cDNA probe used for hybridization was equal to that of the control probe. Hybridization was for 48 h at 42°C in a shaking water bath. Filters were washed three times (15 min each) at room temperature and then three times (1.5 h each) at 68°C, in 0.1X SSC, 0.1% SDS. Filters were blotted and exposed to X-ray films at –70°C, with an intensifying screen, for 16 h and then for 7 d.

Nucleotide Sequencing

The autoradiograms of duplicate blots were compared. Clones that appeared to be kainate-activated in two independent screens were sequenced (ABI automatic DNA sequencer) using T3 and T7 primers for pBluescript. The sequences obtained were compared to all databases and to our own database of CPG sequences.

In Vivo LTP

cDNA clones were randomly selected for LTP tests once their full-length nucleotide sequencing had been completed. LTP was induced in the hippocampal DG granule cells via high-frequency stimulation (HFS) of the perforant path. Male Wistar rats (250–300 g) were anesthetized with Urethane (1.5 g/kg, ip), and surgery and electrophysiological recordings performed in a Kopf stereotaxic apparatus with the plane of the skull oriented horizontally. The stimulation intensity of test pulses (100 µs, 0.066 Hz) was adjusted to yield a population spike amplitude of about 1.0 mV and baseline responses collected for 15 min. Both the initial slope of the positive-going excitatory postsynaptic potentiation (EPSP) and the amplitude of the granule cell population spike were measured. HFS, at an intensity sufficient to elicit the maximum granule cell population spike, consisted of 10 repetitions (1/min) of 50 pulses delivered in 5 bursts, each of 25-ms duration and 400 Hz; bursts were separated by 1 s (Jeffery et al., 1990). DG EEG was monitored before, during, and after each HFS burst. No afterdischarges accompanied the induction of long-term potentiation (LTP) in any of the rats used in this study. Following HFS, the responses to test pulses were monitored for

60 min and the rats sacrificed 30 min, 1 h, 3 h, or 6 h later (two rats at each time-point). Only animals that showed at least a 20% increase in the EPSP slope following HFS were analyzed by *in situ* hybridization. The means of increases in EPSP slope and in population spike amplitude were 35.8 ± 15.6% and 143.8 ± 26.3%, respectively ($n = 8$). The brains were removed and kept frozen at –70°C until sectioning for *in situ* hybridization analysis. Blocking of the NMDA receptor was performed by injection of (RS)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP) (10 mg/kg) 20 min prior to LTP stimulation.

In Situ Hybridization Analysis

Analyses were conducted essentially as described (Hogan et al., 1986). RNA probes were prepared in both sense and antisense orientations using linearized pBluescript SK templates of the CPG cDNAs, and T3 or T7 RNA polymerase. Only clones that showed increased hybridization on the LTP-stimulated side in two rats and at the same time-point were considered LTP-induced.

Construction of a CPG21 Catalytic Site Mutant

Mutant CPG21 (mCPG21) was generated by in vitro mutagenesis, using the oligonucleotide: 5'-GTCCTGGTTCACCTCGAGGCCGGGGTCTCC-3'. For bacterial expression, the full-length open reading frame (1152 bp) was amplified by PCR, using the oligonucleotides: 5'-CCCGAATTCATA TGAAGGTCACGTCGCTC-3' and 5'-CCCAAG CTTGCAGGATGTGGCTGTGGC-3'. The amplified DNA fragment was subcloned into pGEX2T to be expressed and purified as glutathione S-transferase (GST) fusion protein (Guan and Dixon, 1991). For expression in neurons, full-length wild-type and mutant *cpg21* were subcloned through an *Xba*I cloning sites into a mammalian expression vector downstream of an LTR promoter.

Bacterial Expression and In Vitro Assays of CPG21

Bacterially expressed ERK-2 was purified, activated by MEK, in the presence of [γ-^{32}P]ATP and purified on DE-52 minicolumns essentially as described (Seger et al., 1994). The specific activity of ERK-2 was 300 nmol/min/mg. For dephosphorylation reactions, the ^{32}P-labeled ERK-2 (1 µg, 100 µL) was incubated with 100 µL (4 µg) of either CPG21 or its mutant, and dephosphorylation reaction terminated by boiling in gel sample buffer. Proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels that were dried and exposed to an X-ray film. For enzymatic inactivation reactions, a preparation of active ERK-2 (20 ng, 10 µL) was incubated with 10 µL (10 ng) of GST-CPG21, or its mutant form. An aliquot (12.5 µL) was removed after various time intervals and subjected to phosphorylation reaction with myelin basic protein, as described (Seger et al., 1994).

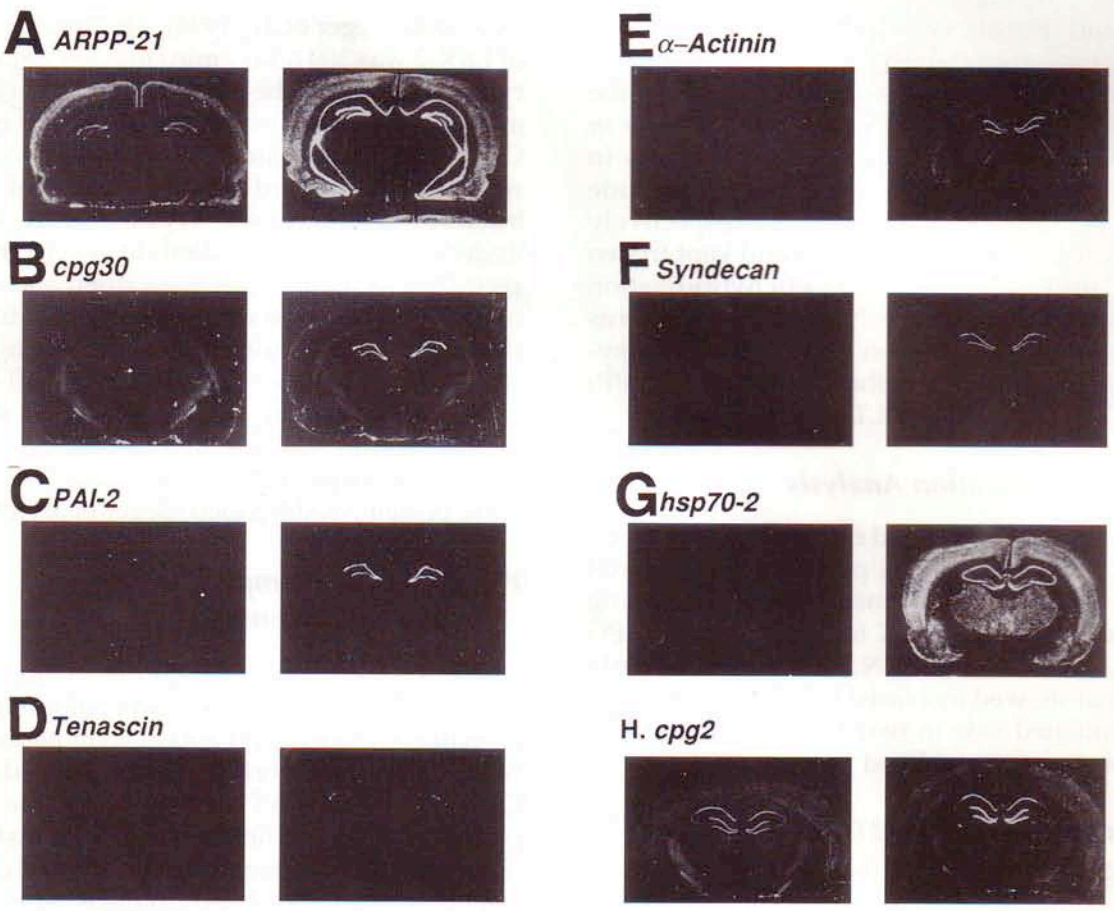
Primary Hippocampal Cultures and Transfections

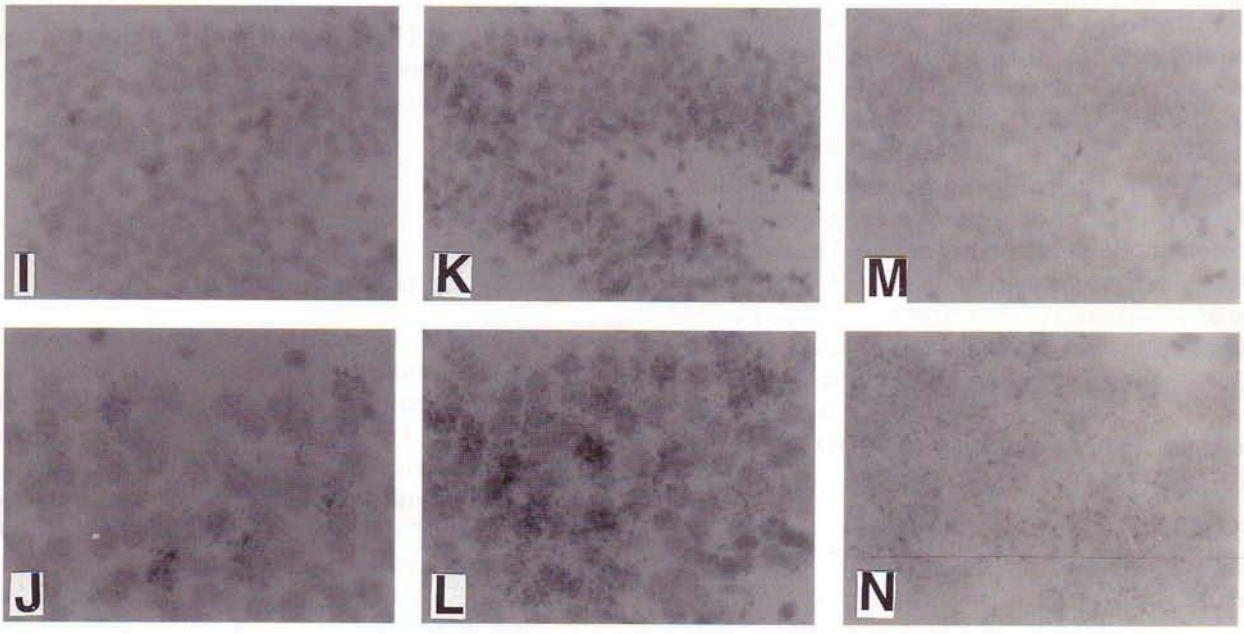
Preparation of primary cultures of hippocampal neurons (E19), transfections, and chloramphenicol acetyltransferase (CAT) assays were performed as we previously described (Rattner et al., 1993). Quantitation of CAT activity was done using a phosphorimager and expressed in histograms showing percent conversion of ^{14}C-labeled chloramphenicol to its acetylated products. Transfections were conducted 1 d after neurons were cultured in vitro. Glutamate (1 m*M*) or MK-801 (2 µ*M*) treatments were for 12 h, beginning immediately after transfection. The *c-fos* promoter plasmids used for transfections were previously described (Graham and Gilman, 1991).

Results

Strategy for Genetic Screen

CPGs were operationally defined as DG transcripts that undergo induction 6 h after ip injection of the potent glutamate analog, kainate (Nedivi et al., 1993). To clone kainate-induced transcripts, we extracted RNA from the DG of treated rats, and then eliminated, through DNA hybridization, transcripts that were not affected by the drug. This enabled construction of a subtracted cDNA library, whose clones were indi-


A ARPP-21
B cpg30
C PAI-2
D Tenascin
E α–Actinin
F Syndecan
G hsp70-2
H. cpg2


I
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vidually screened by differential hybridization to cDNA pools prepared from treated and untreated animals (*see* Experimental Procedures). Over 10^4 clones of the subtracted kainate-activated cDNA library were screened, and the results are summarized in Table 1. Approximately 4.8% of the transcripts that were examined conformed to the criteria of CPGs. Partial nucleotide sequencing indicated that the majority of the isolated CPGs represented unknown genes. Full-length nucleotide sequencing of seven of the novel transcripts provided clues to their function, because they belonged to known protein families or contain recognizable protein motifs (Table 1). In addition, 41 transcripts (termed dCPGs) that underwent downregulation on kainate treatment were isolated. To test directly the functional basis for identification of the isolated CPGs, we compared by *in situ* hybridization the expression patterns of 79 of the transcripts in brains of kainate-treated and untreated animals. Of the 79 clones tested, 68 showed detectable transcripts by *in situ* hybridization. We attribute the lack of detectable signal with the 11 remaining clones to the low abundance of some transcripts cloned by the extremely sensitive "clone-by-clone" method. Examination of 38 clones for transcriptional induction by kainic acid treatment of two animals revealed that most clones were induced. Therefore, the other 30 clones were tested only in one animal. Overall, gene induction was observed with 65 of the 68 detectable transcripts (95%), demonstrating an unusually low percentage of false positives in the differential screen. Figure 1 depicts examples of *in situ* hybridization analyses performed with some representative CPGs. Although each CPG displayed a unique pattern of spatial expression, in all cases, induction in the DG was prominent. High-power magnification localized the induced transcripts in the DG to the granular layer (Fig. 1I–N). Owing to the large size of the CPG group, only ten of the 41 dCPGs isolated in the primary screen were analyzed in one animal by *in situ* hybridization (data not shown). This analysis, which was performed 6 h after kainate treatment, confirmed kainate-induced downregulation of 5 dCPGs at the selected time interval, suggesting that the real number of dCPGs is lower than 41. However, even the maximal number of 41 is considerably less than that of the upregulated CPGs, implying that gene induction, rather than gene repression, mediates neural plasticity.

Classification of the Known CPGs

The known CPGs may be tentatively classified, on the basis of their deduced protein structures, into several main categories (Table 2). The major group is comprised of proteins that are involved in transduction of extracellular signals, and includes neurotrophic factors, membrane receptors, protein kinases and phosphatases, heat-shock proteins, and transcription factors. Another major group encodes structural proteins, that presumably play a role in synapse generation and maintenance. The observed broad pattern of gene activity may represent a pleiotropic cellular response that reflects one or several signaling cascades. Some of the CPGs tested for their kinetics of induction showed a time-course of induction consistent with a sequential genetic response (Fig. 2): The IEG *zif268*, encoding a transcription factor, was induced first, and levels of expression peaked at the earliest time-point tested. *cpg1*, encoding a predicted trophic factor, was induced somewhat later and rapidly returned to basal levels. The protease inhibitor TIMP (16C8) was induced early, but its level of induction was maintained persistently for at least 48 h, consistent with a possible role in synaptic elaboration processes (Monard, 1988).

Fig. 1. (previous) *In situ* hybridization analyses of selected CPGs. **(Top)** The indicated CPG cDNAs were used to synthesize probes for *in situ* hybridization analysis of control (left) and kainate-activated (right) coronal rat brain sections. The identities of the selected CPGs **(A–H)** are as follows: *ARPP-21*, a cAMP-regulated brain phosphoprotein; *cpg30*, a seven-transmembrane span protein; *PAI-2*, an inhibitor of tissue plasminogen activator-2; Tenascin, an extracellular matrix protein; α-actinin, a cytoskeletal protein; Syndecan, a heparin-sulfate proteoglycan core protein; hsp70-2, a heat-shock protein; and *cpg20*, a putative transcription factor related to Zif68. **(Bottom)** Bright-field micrographs of the DG granular layer of sections prepared from kainate-treated rats showing variabilities in the degree of induction. Silver grains represent *in situ* hybridization patterns specific for *Tenascin* **(I, J)**, *PAI-2* **(K, L)**, and *cpg20* **(M, N)** photographed at magnifications of 10X (I, K, and M) or 20X (J, L, and N).

Table 2
Known CPGs and Their Possible Relationship to Plasticity[a]

CPG	Reference
IEGs	
Transcription factors	
***c-jun**	Kitabayashi et al., 1990
junB	Kawakami et al., 1992
***zif268**	Sheng and Greenberg, 1990
***c-fos**	Curran et al., 1987
***fosB**	Zerial et al., 1989
**CREM*	Foulkes et al., 1991
***BTE**	Imataka et al., 1992
***Nur77**	Ohkura et al., 1994
Nor-2	Petropoulos et al., 1995
***cpg20*, **E4BP4*	Cowell et al., 1992
***EGR3**	Patwardhan et al., 1991
Siah	Della et al., 1993
**NGFI-C*	Crosby et al., 1991
LRF-1	Hsu et al., 1991
RNR-1	Scearce et al., 1993
**TIS11*	Kaneda et al., 1992
IkB	Tewari et al., 1992
Heat-shock proteins	
**cpg4*, **hsp27*	Gaestel et al., 1993
**hsp70* and **hsp70-2*	Powell and Watts, 1990
Trophic factors and signaltransduction proteins	
Trophic factors	
**cpg1*	Nedivi et al., 1993
**MARC*	Kulmburg et al., 1992
**MCP-1*	Yoshimura et al., 1991
***BDNF**	Maisonpierre et al., 1990
Activin	Fang et al., 1996
**PC3*	Bradbury et al., 1991
PEDF	Steele et al., 1993
Signal transduction	
***MyD118**	Abdollahi et al., 1991
**ME491*	Rapp et al., 1990
***ARPP-21**	Ehrlich and Greengard, 1991
Metallothionein	Andersen et al., 1986
**rheb*	Yamagata et al., 1994b
**STAT3*	Ripperger et al., 1995
**IGF-BP3*	Shimasaki et al., 1989
**RC3*	Watson et al., 1990
**S-adenosylmethionine decarboxylase*	Pajunen et al., 1988
**Calbindin/Neuronatin*	Lomri et al., 1989
Protein phosphatases	
**PTP1B*	Guan et al., 1990
**cpg21 , BSM-1*	accession number D45412
Protein kinases	
**cpg16*, **FLT*	Finnerty et al., 1993
**PKR*	Mellor et al., 1994
Retrograde messengers	
***Cox2 cyclooxygenase**	O'Banion et al., 1992
**Heme oxygenase*	Muller et al., 1987
**Arginino succinate lyase*	Amaya et al., 1988
Genes possibly involved in synaptic remodeling	
Synaptic transmission-related	
**dyn*	Civelli et al., 1985
PE	Noda et al., 1982
**Clathrin*	Kirchhausen et al., 1987
hsc70	Chappell et al., 1986
**Secretogranin*	Fischer et al., 1990
COMT	Salminen et al., 1990
cpg22, a PDZ domain protein related to Homer	Brakeman et al., 1997
cpg30*, a new 7-TM receptor, **cpg38*, a new 7-TM receptor, ***VGF	Salton, 1991
*nAchR-ε	Criado et al., 1988
**β-DHPR*	Pragnell et al., 1991
Structural proteins	
**cpg2*, a putative new cytoskeletal protein, **Syndecan*	Kojima et al., 1992
Ryudocan	Kojima et al., 1992
***α-actinin**	Youssoufian et al., 1990
**Tenascin*	Saga et al., 1991
α-B-crystallin	Bhat et al., 1991
**Fibrilarin*	Turley et al., 1993
**NF-L*	Julien et al., 1985
***Arc/Arg3.1**	Link et al., 1995
	Lyford et al., 1995
**SC2*	Johnston et al., 1992
**ABGP*	Davis et al., 1993
Protease inhibitors	
**16C8*	Edwards et al., 1986
**PAI-2*	Antalis et al., 1988
DNA replication	
**DNA pol.β*	Zmudzka et al., 1986
	Yamaguchi et al., 1987
HMG2	Majumdar et al., 1991

[a]Clones analyzed for induction by LTP are indicated by asterisks and those that scored positive appear in bold.

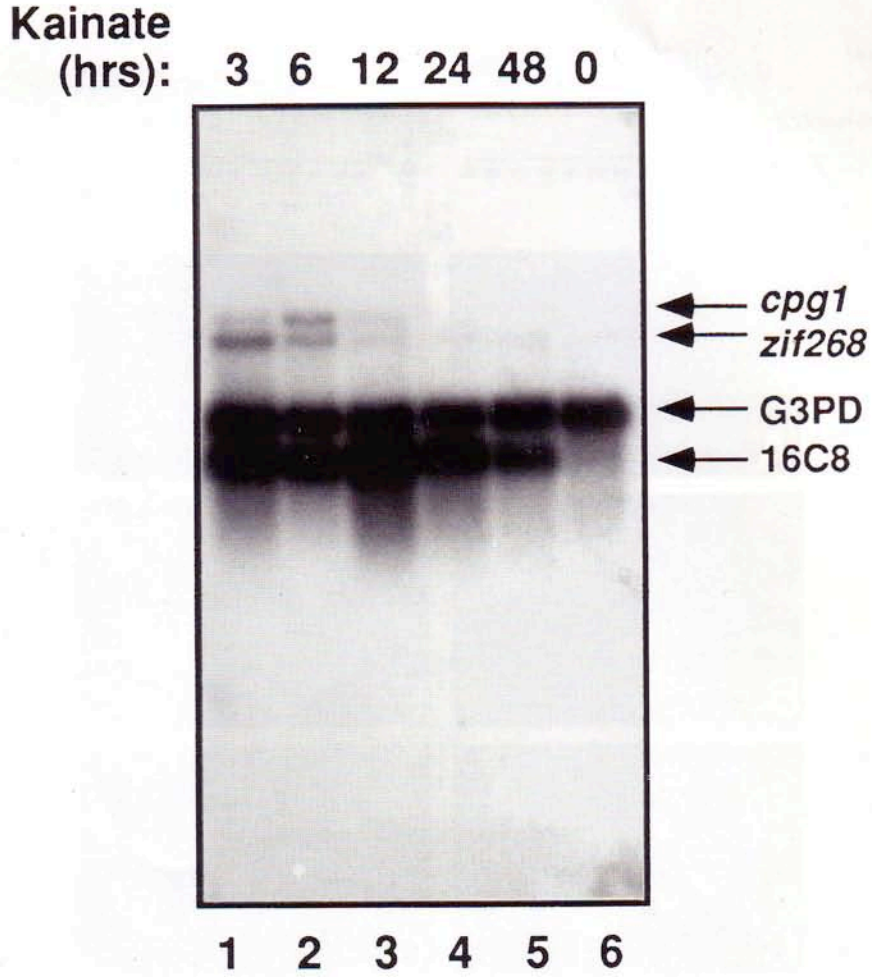

Fig. 2. Kinetics of kainate induction of three CPGs. DG poly(A)$^+$ RNA (0.5 μg) from control (lane 6) or kainate-treated rats, that were sacrified at the indicated time after treatment, was used for Northern blot analysis. The filter was first hybridized to a control probe, glyceraldehyde-3-phosphate-dehydrogenase (*G3PD*), and then to a mixture of probes specific to the following three genes (*see* Table 2): *cpg1*, *zif268*, and *16C8*. Similar analyses were performed with additional CPGs and induction was found to be either transient (e.g., c-*jun*, c-*fos*, *MyD118*, *hsp27*, and *PTP1B*), intermediate (e.g., *cpg4*, *CREM*, and *Clathrin*), or persistent (e.g., *Secretogranin*, *cpg2*, and *Dyn*).

Importantly, kainate activation of the DG not only induced, but also repressed gene expression. Out of the 41 downregulated genes (dCPGs) that were cloned and sequenced, only five are known genes. Notably, *in situ* hybridization confirmed that one of the isolated dCPGs encodes a glutamate receptor subunit (kainate-KA1). This finding probably reflects a mechanism of protection from overstimulation by kainate. Interestingly, two CPGs encode postsynaptic excitatory proteins (the nicotinic acetylcholine receptor, nAChR-ε, and the β-subunit of the brain dihydropyridine-sensitive calcium channel, β-DHPR; Table 2).

CPG Induction by an LTP-Producing Stimulus

LTP is an important model of memory-related synaptic plasticity (Bliss and Collinridge, 1993). To address the relevance of CPGs to LTP, 58 of the 74 CPGs listed in Table 2 (marked by asterisks), as well as 8 additional novel CPGs, were tested for induction by an LTP-producing stimulus in vivo (Fig. 3, and data not shown). Of this group, 17

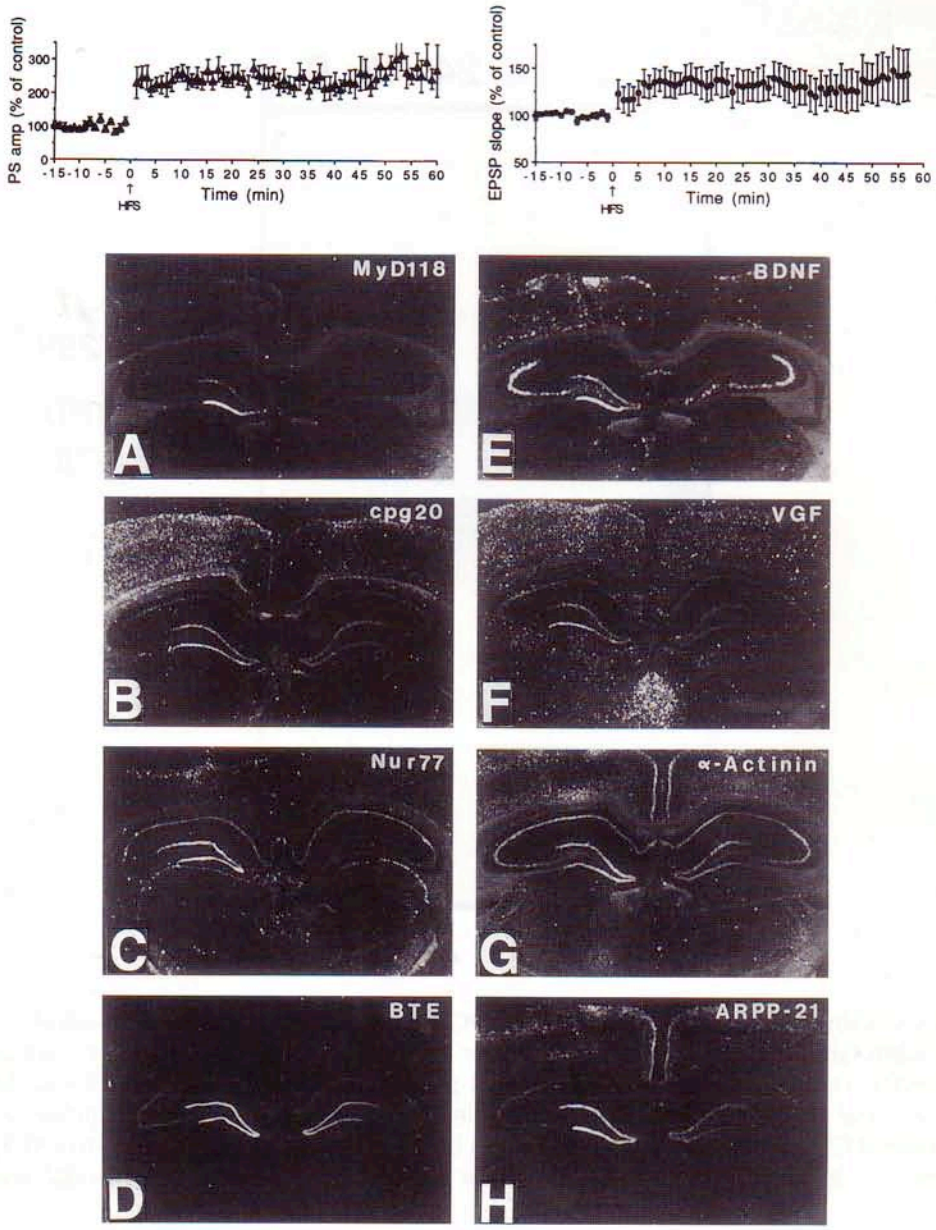

Fig. 3. CPG induction by LTP-producing stimuli. **(Top)** Potentiation of field potential responses is shown as averaged data (n = 6) recorded in the granular layer of the dentate gyrus. Population spike (PS) amplitude (triangles) and initial EPSP slope (closed circles) are expressed as a percentage of the mean baseline responses before application of high-frequency stimulation (HFS) to the perforant path (marked by arrows at time zero). **(Bottom)** *In situ* hybridization analyses of representative CPGs. LTP was conducted on the left hemisphere, whereas the right hemisphere served as a control. Data presented are from rats sacrified 1 h following HFS. Brain slices were hybridized with riboprobes specific for the indicated CPGs (*see* Table 2).

CPGs (15 marked by bold letters in Table 2, and 2 novel CPGs) exhibited greater induction in the stimulated (left side of the hippocampus; Fig. 3), than in the contralateral hippocampus. All of these

LTP-induced transcripts displayed elevated levels 1 h after HFS, and some maintained high levels for as long as 6 h after stimulation (data not shown). In order to confirm that the observed induction by LTP occurs through activation of the *N*-methyl-D-aspartate (NMDA) receptor, control rats were treated with the NMDA receptor blocker CPP prior to LTP stimulation. Seven CPGs (*MyD118*, *cpg20*, *BDNF*, *EGR*, *VGF*, *ARPP21*, and *c-jun*) were tested by *in situ* hybridization, and none of them exhibited a significant induction after CPP injection (data not shown), in accordance with the prediction that CPG induction by LTP depends on an active NMDA receptor.

Novel CPGs: A Putative Transcription Factor and a Protein Kinase

Complete nucleotide sequences were determined for 15 of the cloned novel CPGs. The deduced protein sequences of 7 of the 15 CPGs contained sufficient homologies to known proteins, thereby enabling prediction of their cellular functions (Table 2 and Fig. 4). These are *cpg2*, a dystrophin-like cytoskeletal protein, *cpg4*, a presumed heat-shock protein, *cpg20*, a potential transcription factor, *cpg16*, a putative protein kinase distantly related to calcium and calmodulin-(CaM)-dependent kinases, *cpg21*, a predicted protein phosphatase related to mitogen-activated protein kinase (MAPK) phosphatases, and two putative receptors, each containing seven-transmembrane domains, that are encoded by *cpg30* and *cpg38*. A human homolog of CPG30 has been recently identified as a pituitary receptor involved in growth hormone release (Howard et al., 1996).

To exemplify their relationships to known protein families, the primary structures of three novel CPGs are compared with their closest relatives (Fig. 4). The full-length amino acid sequence of CPG20 predicts a new protein with three zinc fingers homologous to the transcription factors Sp-1 and Zif268/Egr-1 (boxed in Fig. 4A). The latter is probably the most thoroughly studied gene that responds to LTP-producing stimuli (Cole et al., 1989; Silva and Giese, 1994). Similarly, we observed that *cpg20* undergoes upregulation by an LTP-producing stimulus (Fig. 3B). However, the zinc fingers of CPG20 are more related to those of Sp-1 than to the fingers of Zif268/Egr-1, and all residues determining DNA sequence specificity are shared, suggesting that CPG20 and Sp-1, but not Zif268/Egr-1, bind to similar or identical sequence elements of genomic DNA. Recently, two possible human orthologs of *cpg20* were isolated as growth response transcripts (Blok et al., 1995; Subramaniam et al., 1995).

In situ hybridization analyses, which are not presented, indicated that *cpg16* is transcriptionally active in several brain regions, including the cortex and the hippocampus. The primary structure of CPG16 predicts a protein kinase function, because all of the 11 subdomains shared by the protein kinase superfamily (Hanks, 1991) are conserved in CPG16 (Fig. 4B). Specifically, CPG16 is more closely related to ser/thr-specific kinases than to tyrosine-specific protein kinases. Although, the closest relatives of CPG16 are all members of the calcium/calmodulin-dependent protein kinases (CaM kinases, including CaM kinases I and II, and myosin light-chain kinase), the C-terminally-located calmodulin binding region of established CaM kinases (Maedor et al., 1993) differs from the corresponding region of CPG16 (Fig. 4B). Indeed, experiments performed with a bacterially expressed CPG16 fusion protein indicated that CPG16 may not function as an ordinary CaM kinase (M. A. S. and R. S., manuscript in preparation): First, the protein did not bind to an agarose-immobilized calmodulin. Second, although CPG16 undergoes autophosphorylation in vitro, as expected for many ser/thr-specific kinases, its activity was not affected by two CaM kinase inhibitors, calmidazolium and W-7. Finally, in vitro assays have shown that the catalytic activity of CPG16 is independent of calcium and calmodulin, and when expressed in COS-7 cells, a calcium ionophore exerted no effect on the enzymatic activity of CPG16. In conclusion, despite structural relatedness, CPG16 is apparently regulated by a mechanism distinct from that controlling the enzymatic function of CaM kinases.

CPG21, a MAP-Kinase Phosphatase Capable of Transcription Regulation

Transcripts of *cpg21* are undetectable in rat brain, except for the CA2 region of the hippocampus. Treatment with kainaic acid elevated transcription of *cpg21* in the DG, CA2, and CA1 regions (data not shown). The deduced amino acid

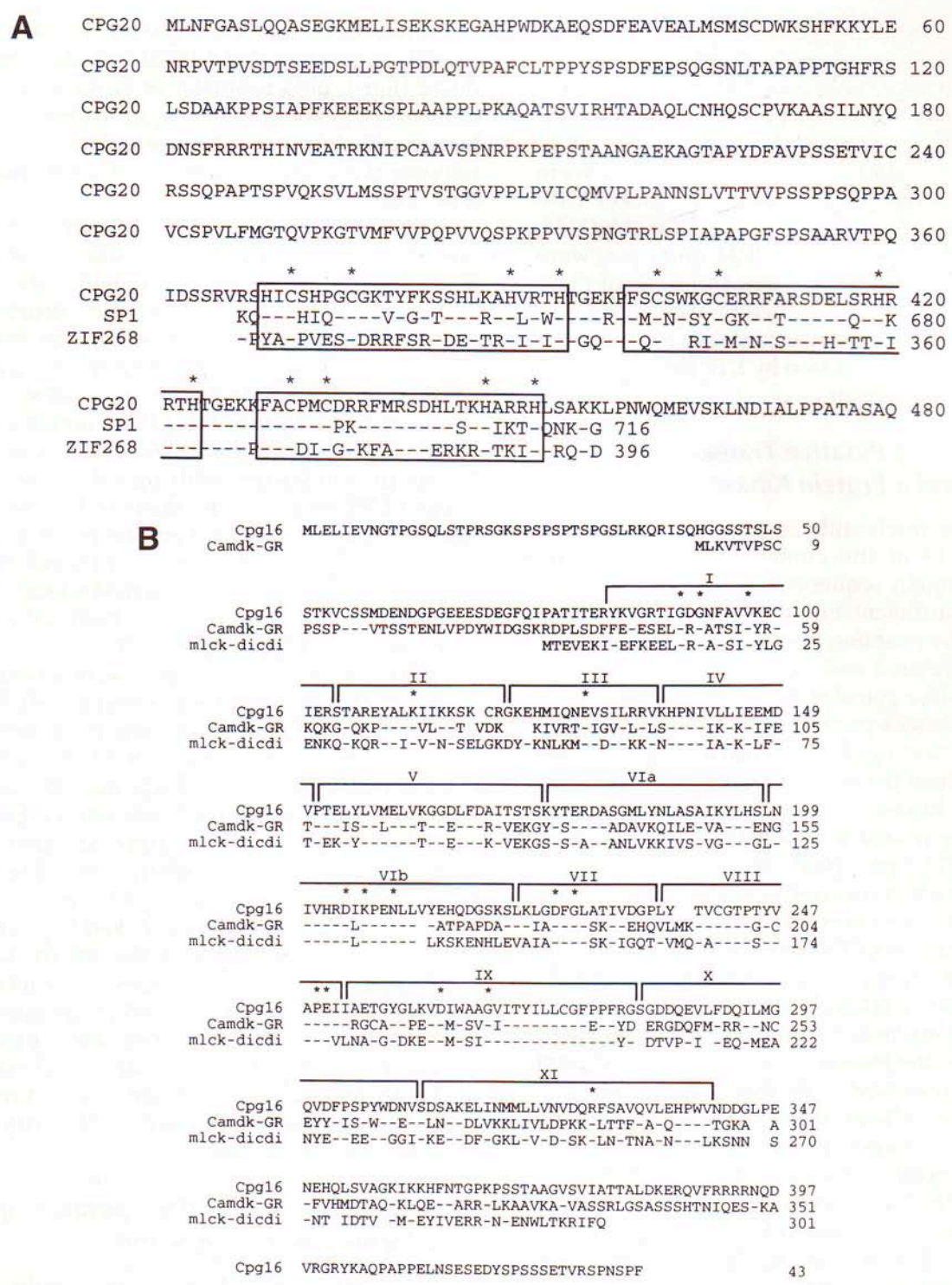

Fig. 4. Predicted amino acid sequences of selected CPGs and comparisons with their closest homologs. Dashes indicate identical residues; gaps were introduced for maximal alignment. **(A)** CPG20, a zinc-finger protein of the Sp1/Egr family. The full-length nucleotide sequence of the *cpg20* cDNA was determined, and the predicted amino acid sequence is shown. The boxed sequences indicate the three zinc fingers of CPG20, and their sequences are *(continued)*

C

```
CPG21          MKVTSLDGRRLRKMLRKEAEA RCVVLDCRPYLAFAASSVRGSLNVNLNSVVL  52
hVH3           M-----------------A- ----------------N-------------  52
MKP-1      MVMEVGI --AGG--AL--ER  -AQ-LL----SFF--N-GHIV--V--RFSTI-R  53
PAC-1  MPIAMGLETACE-ECAA-GAL--EPR--E-TLL-----F---CRSH-RAARP-PW-ALLR  60

CPG21  RRARGG AVSARYVLADEAARARLLQEGGGGVAAVVVLDQGSRHWQKLREESAARVVLTS  111
hVH3   ------ --------P-------------------------------------------  111
MKP-1  ---K-  -MGLEHIVPNTEL-G---A   -AYTP--L--ER-AALDGRKRDGTLALAAGA  108
PAC-1  ---P-TP-AALACL-P-R-L----GR   -EL-RA----ES-ASVTE-PPDGPAHLL-AA  117

CPG21  LLACLSAGP RVYFLKGGYETFYSQYPECCVDAKPISQEKLEGERGLLSQCGKPILSVAY  170
hVH3   -----P--- --------------E-------V-------I-S--A-I------VVN-S-  170
MKP-1  -CREARS  TQ-F--Q----A-SASC--L-SKQSTPMGLS- PLSTSVPDSAESGC-SCS  165
PAC-1  -QHEMRG--TT-C--R--FKS-QTYC-DL-SE-PAQ   A- P     PAGAENSN-DPR  168

CPG21  RPAYDQGGPVEILPFLYLGSAYHASKCEFLANLHITALLNVSRRTSEACTTHLHYKWIPV  230
hVH3   ----------------------------------------------M-----------  230
MKP-1  T-L----------S-----------RKDM-DA-G----I---ANCPNHFEG-YQ--S---  225
PAC-1  V-I-----------Y-----CN-S-DLQG-QACG---V----ASCPNHFEGLF---S---  228

mCPG21                                 L
CPG21  EDSHTADISSHFQEAIDFIDCVREEGGKVLVHCEAGVSRSPTICMAYLMKTKQFRLKEAF  290
hVH3   ----------------------K---:::C---I-----------------------  290
MKP-1  --N-K-----W-N-------SIKDA--R-F---CQ---I---A---L----R-NRVK-D---  285
PAC-1  --NQMVE--AW-----S---S-KNS--R-:::CQ--I---A---L---IQSHRV--D---  288

CPG21  EYIKQRRSVVSPNFGFMGQLLQYESEILPSTPTPQPPSCQGEAASSTFIGHLQTLSPDMQ  350
hVH3   D-------M----------------------N-----------G-SL------------  350
MKP-1  -FV-----II----S-------F--QV-       A-H-SA--G-PAMAVLDRGT-TT    336
PAC-1  DFV----G-I----S-------L-TQV-       CH                         318

CPG21  GAYCTFPTSVLAPVPTHATVAELHRSPVATATSC                             384
hVH3   -------A---------S--S--S----------                             384
MKP-1     TV-NFP-SI--HPTNSALNYLQ--IT-SP--                             367
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Fig. 4. *(continued)* compared to the corresponding zinc fingers of the proteins Sp1 and Zif268 (also called Egr-1). Asterisks denote the conserved cysteine and histidine residues of the zinc fingers. **(B)** CPG16, a novel kinase related to serine- and threonine-specific protein kinases. The deduced amino acid sequence of CPG16 is compared to its closest kinases: the calcium/calmodulin-dependent protein kinase Gr (Ohmstede et al., 1991), and the Dictyostelium discodeum myosin light-chain kinase (mlck-dicdi). Subdomains of CPG16 shared with other kinases (numbered I–XI) (Hanks, 1991) are indicated. Asterisks mark amino acids conserved through the whole family of kinases. Residue numbers are indicated in the right column. **(C)** CPG21, a protein phosphatase. The deduced amino acid sequence of CPG21 is aligned with the following PTPases: hVH3 (human) (Kwak and Dixon, 1995), MKP-1 (Keyse and Emsile, 1992; Sun et al., 1993), and PAC-1 (Ward et al., 1994). Residue numbers are shown in the right column. The PTPase signature sequence is indicated (stippled box) and the Cys (bold) to Leu active site mutant of CPG21 (*mCPG21*) is shown.

sequence of CPG21 predicts a 384-residue-long protein with a high degree of homology to two human dual-specificity protein phospatases, bVH3 (94% identity) and B23 (84% identity) (Kwak and Dixon, 1995). The two human proteins differ in their C-termini, but they share a cysteine-containing canonical phosphatase motif that slightly differs from that of CPG21 (Fig. 4C, and data not shown). This signature motif, as well as additional blocks of conserved sequences, are common to other protein tyrosine phosphatases (PTPases), including two related dual-specificity phosphatases that dephosphorylate and inactivate MAPK, namely MKP-1 (Keyse and Emsile, 1992; Sun et al., 1993) and PAC-1 (Ward et al., 1994) (approx 45% identity).

In order to examine the predicted phosphatase activity of CPG21 and its expected specificity to MAPK, we expressed a full-length cDNA of *cpg21*

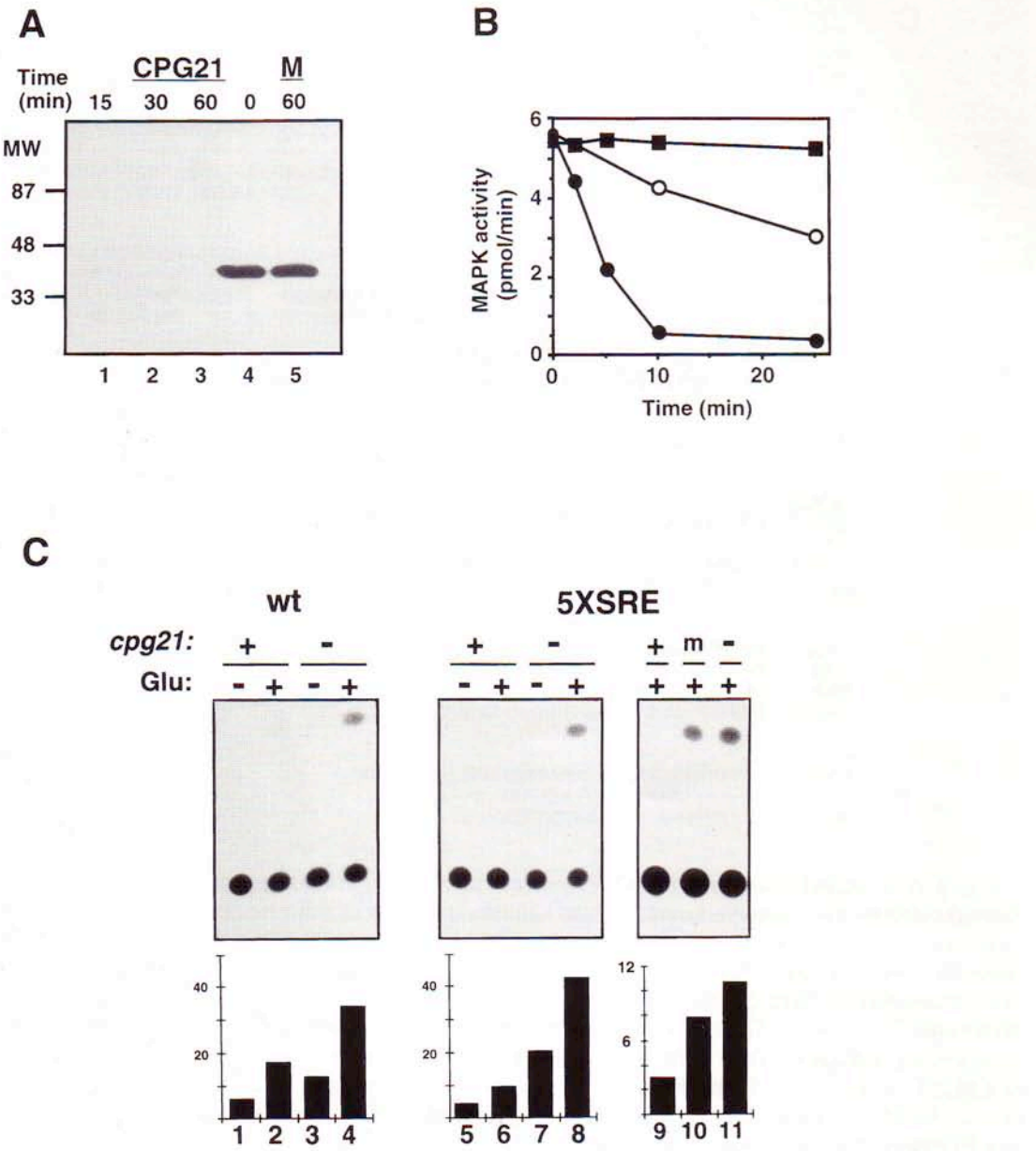

Fig. 5. Functional analyses of CPG21 in vitro and in living cells. **(A)** Dephosphorylation of MAPK. Bacterially expressed MAPK was phosphorylated in vitro by using [γ-^{32}P]ATP and MAPK-kinase (MEK). The radioactively labeled MAPK was incubated with a bacterially expressed wild-type CPG21, or with an active site mutant (m), for the indicated time intervals, and the reaction mixtures resolved by gel electrophoresis (mol-wt markers are indicated in kilodaltons), followed by autoradiography. **(B)** Inactivation of MAPK. MAPK (ERK-2) was phosphorylated by MEK as in A, except that nonradioactive ATP was used. The active enzyme was purified over an ion-exchange minicolumn and incubated at 37°C with either a GST-CPG21 fusion protein (closed circles), or with an active site mutant (squares) for the indicated time intervals. For control, the wild-type fusion protein was incubated with 5 m*M* vanadate (open circles). Kinase assays were performed at the end of incubation by using myelin basic protein as a substrate. **(C)** Inactivation of SRE-mediated transcription in hippocampal cells. Primary cultures of rat hippocampal cells (embryonic d 19) were transfected with a *c-fos* promoter construct (1 µg/dish), either a plasmid containing a 356-bp-long 5'-sequence of *c-fos* (marked wt), or a plasmid containing 5 copies of SRE (*5XSRE*) fused to a (continued)

in bacteria and tagged it with a GST moiety. As control, we attempted to block the enzymatic activity by introducing a cysteine to leucine replacement in the catalytic core of CPG21 (Fig. 4C). When incubated with a phosphorylated MAPK (ERK-2), purified preparations of CPG21, but not its mutated version (denoted mCPG21), rapidly dephosphorylated the kinase (Fig. 5A). Because MAPK phosphorylation was performed by using MEK, an MAPK kinase that activates MAPK, dephosphorylation by CPG21 is expected to inactivate MAPK. Indeed, a bacterially expressed GST-CPG21 fusion protein inhibited the ability of MAPK to phosphorylate myelin basic protein in vitro (Fig. 5B). As expected, the catalytically impaired mutant of CPG21 exerted no effect on MAPK activity (Fig. 5B). Vanadate, a potent tyrosine PTPase inhibitor, partially abolished the effect of CPG21, consistent with the possibility that CPG21 functions as a dual-specificity PTPase.

Interestingly, similar in vitro assays that tested the ability of CPG21 to inactivate the MAPK-related Jun-N-terminal kinase (JNK or SAPK) detected no effect on the ability of purified JNK1 to phosphorylate a recombinant Jun protein in vitro (data not shown). Taken together, the assays performed with CPG21 indicated that this protein can act in vitro as a MAPK-specific phosphatase, whose catalytic site shares similarity with the related MKP-1 and PAC-1 PTPases (Sun et al., 1993; Ward et al., 1994).

We next attempted to examine the effect of CPG21 on MAPK in living cells. As an experimental system, we selected primary cultures of hippocampal neurons, because glutamate activation of the NMDA receptor in these cells was shown to cause phosphorylation of MAPK (Bading and Greenberg, 1991) and induction of *c-fos* expression (Bading et al., 1993), through the serum response element (SRE) of its promoter. The adequacy of the hippocampal culture system was confirmed in initial studies, whose results are not presented, demonstrating CPG21 expression only on treatment with glutamate. In addition, we found that *cpg21* induction was preceded by upregulation of *c-fos*, and it could be blocked by the NMDA receptor blocker MK-801.

Two previously described CAT reporter constructs (Graham and Gilman, 1991) were employed to follow SRE-mediated control of *c-fos* expression by CPG21: a 356-bp-long upstream region of *c-fos* containing a single SRE (denoted wild-type, wt), and another construct carrying five copies of SRE in front of a 56-bp-long minimal promoter of *c-fos* (5XSRE). Both plasmids, when transfected into hippocampal neurons, induced basal expression of the reporter CAT enzyme. Cell treatment with glutamate significantly enhanced CAT expression (Fig. 5C), confirming coupling of NMDA receptors to SRE (Bading et al., 1993). However, cotransfection of hippocampal cells with an expression vector driving *cpg21* reduced basal, as well as glutamate-induced expression of CAT from either a WT *c-fos* promoter or the 5XSRE construct (Fig. 5C). An expression vector encoding a catalytically inactive CPG21 exerted only a small inhibitory effect on glutamate-induced expression from the 5XSRE construct (Fig. 5C), in line with a model attributing transcription control to inactivation of MAPK-mediated regulation of SRE.

It is relevant to note that the inhibitory activity of CPG21 was more prominent when 5XSRE, rather than a full-length promoter, was used as a reporter (Fig. 5C). In addition, inhibition by CPG21 was observed even when the NMDA receptor pathway was blocked by MK-801. Together with the incomplete shutdown of transcription by CPG21, these observations may reflect an ability of CPG21 to block also non-NMDA receptor-coupled pathways. In addition, these results suggest involvement of elements other than SRE in *c-fos* induction by glutamate. Nevertheless, the relatively late induction of *cpg21* (induction is observed after *c-fos* expression is reduced to basal level, data not shown) and its biochemical functions, implicate this novel PTPase in a mechanism that downregulates neural responses to excessive glutamate-induced gene expression.

56-bp-long 5' sequence of *c-fos*. In both plasmids, a CAT coding sequence was fused to the promoter region. Where indicated, cultures were cotransfected with either an expression vector encoding CPG21 (5 μg/dish, lanes 1, 2, 5, and 6, or 9 μg in lane 9), or an active site mutant (marked m, 9 μg), and cells were treated with either glutamate (1 m*M*, lanes marked +), or the NMDA receptor blocker MK-801 (5 μ*M*, lanes marked -), immediately following plasmid transfection. CAT activity was determined 12 h later by the ability of the corresponding cell extracts to acetylate labeled chloramphenicol. Results are expressed in arbitrary units (lower panels). The experiment was repeated three times.

Discussion

Conventional differential cDNA cloning may allow identification of candidate genes involved in producing phenotypic changes. The major problem of this approach is sensitivity; genes that are not highly expressed may evade detection. By contrast, the "clone-by-clone" strategy yielded a large number of differentially expressed genes. Two features of this strategy are prominent (Table 1): First, high sensitivity of detection; almost 80% of the library's recombinants produced a signal in the differential screen. Second, the strategy enables high yield of differentially expressed independent cDNA clones. This large number is probably owing to the high sensitivity of the screen, and to faithful representation of the DG RNA within the subtracted library.

Regardless of its possible technological importance, the present study may be significant to the molecular mechanisms that underlie neural plasticity. In this context it is relevant to ask whether all the identified CPGs are involved in plasticity, or if they mediate disparate processes that merely reflect glutamate excitotoxicity (Ben-Ari, 1985). For example, plasticity and excitotoxicity were shown to activate the same set of genes (e.g., c-*fos*, c-*jun*, *zif268*) (Smeyene et al., 1993; Dragunow et al., 1994). In addition, plasticity and cell death are two overlapping processes that take place during CNS development (Shatz, 1990; Raff et al., 1993). The following lines of evidence support involvement of a significant fraction of the isolated CPGs in plasticity, rather than in glutamate excitotoxicity:

1. Several CPGs identified by the present screen were previously associated with plasticity. These include IEGs, genes encoding neurotrophins, neuropeptides, signal transduction proteins, and dendritic components (Yamagata et al., 1993, 1994a,b; Silva and Giese, 1994; Lyford et al., 1995).
2. A subset of six CPGs are specifically induced in visual cortex in response to light, and are also naturally induced during postnatal cortical development (Nedivi et al., 1996). One of these, CPG15/neuritin, encodes a glycosylphosphatidylinositol-anchored protein that promotes neurite outgrowth in primary embryonic hippocampal cultures (Naeve et al., 1997), and enhances dendritic arbor growth of tectal cells in vivo (Nedivi et al., 1997). Another, CPG22, is a PDZ protein related to Homer, a dendritic protein that selectively binds metabotropic glutamate receptors (Brakeman et al., 1997).
3. CPG induction appears to be localized primarily to the granular cells of the DG, rather than to basket or glial cells (note that in Fig. 1, tenascin is the exception, being one of the few CPGs induced in glia). Since granular neurons are resistant to kainate toxicity (Nadler, 1978; Ben-Ari, 1985), it is likely that CPG induction in these neurons is not related to excitotoxicity, but rather to the propensity of granular cells to exhibit massive mossy fiber sprouting, as well as physiological changes, on kainate activation (Tauck and Nadler, 1985; Ben-Ari and Represa, 1990; Sloviter, 1992).
4. Seventeen out of 66 CPGs that were tested undergo upregulation in response to an LTP-producing stimulus (Fig. 3).

We find it especially important that a significant proportion of the identified CPGs undergo induction during LTP. This fraction is likely to be a minimal estimate because LTP was induced under anesthesia that partially blocks glutamate receptor activation (Demmer et al., 1993; Frank and Greenberg, 1994). Moreover, the following six CPGs: *BTE*, *cpg20*, *MyD118*, *ARPP-21*, *VGF*, and α-actinin are described here for the first time as genes coinduced with LTP in vivo. Notably, not only regulatory genes, such as *zif268* or *cpg20*, but also genes encoding structural proteins, such as α-actinin, the vesicle protein VGF (Possenti et al., 1989), and the dendritic protein Arc/Arg3.1 (Link et al., 1995; Lyford et al., 1995), are induced by LTP-producing stimuli. This supports the notion that morphological alterations play a role in longer-lasting LTP (Bailey and Kandel, 1993; Bliss and Collinridge, 1993; Huang et al., 1994).

Owing to its unbiased nature, our screening strategy may identify molecular mechanisms whose participation in neural plasticity is currently not fully appreciated. Examples include the putative dual-specificity phosphatase encoded by *cpg21* and a newly described protein kinase, CPG16, whose closest relatives are CaM kinases and a myosin light-chain kinase. Both CaM kinase II and protein phosphatases have been implicated in LTP and in long-term depression (LTD) (Silva et al., 1992; Mulkey et al., 1994; Thomas et al., 1994; Mayford et al., 1995). However, functional analyses of CPG21 imply that this kinase is

regulated by cAMP levels, rather than by the calcium/calmodulin axis (M. A. S. and R. S., manuscript in preparation). Nevertheless, the nature of the majority of CPGs appears to be consistent with existing models of neural plasticity (Goelet et al., 1986; Turley et al., 1993; Thomas et al., 1994). What follows is a brief description of these models and their possible relationships to CPGs (Fig. 6). Not included are signal transduction molecules, such as transcription factors and cytoplasmic signaling molecules, since these may contribute to all models.

1. Synaptic elaboration—pre- or postsynaptic elaboration is expected to change patterns and strengths of synaptic connections (Bailey and Kandel, 1993; Bliss and Collinridge, 1993; Huang et al., 1994). Accordingly, most of the isolated CPGs fall into this category, including several trophic factors (e.g., BDNF and activin), cyto-skeletal and other structural proteins (e.g., syndecan, Arc/Arg3.1, α-actinin, and fibrillarin), and two protease inhibitors (TIMP and PAI-2).
2. Presynaptic release—changes in the amount of released neurotransmitter would affect synaptic efficacy (Bekkers and Stevens, 1990; Bliss and Collinridge, 1993; Arancio et al., 1995). The two new seven-transmembrane domain receptors (CPG30 and CPG38) could be involved in modulation of the amount of transmitter released in a manner similar to that of other modulatory receptors (Aramori and Nakanishi, 1992). Up-regulation of the vesicle proteins clathrin, HSC70, secretogranin, and VGF, which were identified as CPGs, may lead to an increased number of secretory vesicles and thereby elevate quantal release.
3. Postsynaptic receptors—changes in the number or properties of post-synaptic receptors and channels can affect synaptic plasticity (Davies et al., 1989; Manabe et al., 1992; Bliss and Collinridge, 1993). The ε-subunit of the nicotinic acetylcholine receptor and the L-type Ca^{2+} channel subunit, βDHPR, which we found to be upregulated by neural activity, may be involved in modulation of synaptic action.
4. Retrograde messengers—evidence for "retrograde" communication between post- and presynaptic neurons during synaptic processes has been reported (Bliss and Collinridge, 1993; Malenka, 1994). Three major candidates were proposed as retrograde messengers (Williams et al., 1989; Stevens and Wang, 1993; Zhuo et al., 1993): arachidonic acid (AA) and the gases NO and CO. Interestingly, three of the identified CPGs encode enzymes involved in AA metabolism, CO synthesis, and arginine (a precursor of NO) metabolism.

In view of the unexpectedly large number of transcripts that were identified as CPGs, it is relevant to ask how many genes are involved in the response to kainate. In our previous study (Nedivi et al., 1993), this was estimated to be in the range of 500–1000. The present study verifies this prediction, because after screening 10,000 library clones, 362 CPGs, and 41 dCPGs, a significant fraction (~30–50%) of the predicted sum have already been cloned. Similarly, our LTP tests predict that the number of CPGs involved in LTP is in the range of 100–200 genes, implying extensive gene induction. By contrast, only 5–10% of the genes that were modulated by glutamate receptor activation underwent downregulation (termed dCPGs). Of these, five are known genes, including a subunit of a kainate receptor (Gall, 1988), and two are Ca^{2+} binding proteins, S100β and calmodulin. Downregulation of these genes could reflect a protective response of the granular neurons to strong kainate activation. Interestingly, the dCPG group includes only two transcription factors, one of which is the p50 subunit of NF-κB (Liou and Baltimore, 1993). Moreover, we identified the gene encoding the inhibitory subunit (IκB) of NF-κB as a CPG. It is worth noting that NF-κB is a potent activator of gene expression in primary cultures of hippocampal neurons (Rattner et al., 1993). Possibly, this dual regulation may ensure kainate-induced reduction in NF-κB activity. It will be interesting to see whether the dCPGs are genes that require NF-kB activity for expression. Regardless of the identities of dCPGs, one obvious conclusion is that gene activation, rather than repression, is the more prevalent response to the strong activation of the glutamate receptor, which we employed in this study.

Obviously, an unequivocal assignment of any given CPG to neuronal plasticity will require further experiments, involving different and complementing approaches. However, expression in the exci-totoxicity-resistant granular neurons, induction of a substantial portion of the identified CPGs by both LTP-producing stimuli (Fig. 3), and under physiological conditions such as visual excitation (Nedivi et al., 1996), together with the nature of the known CPG products, are

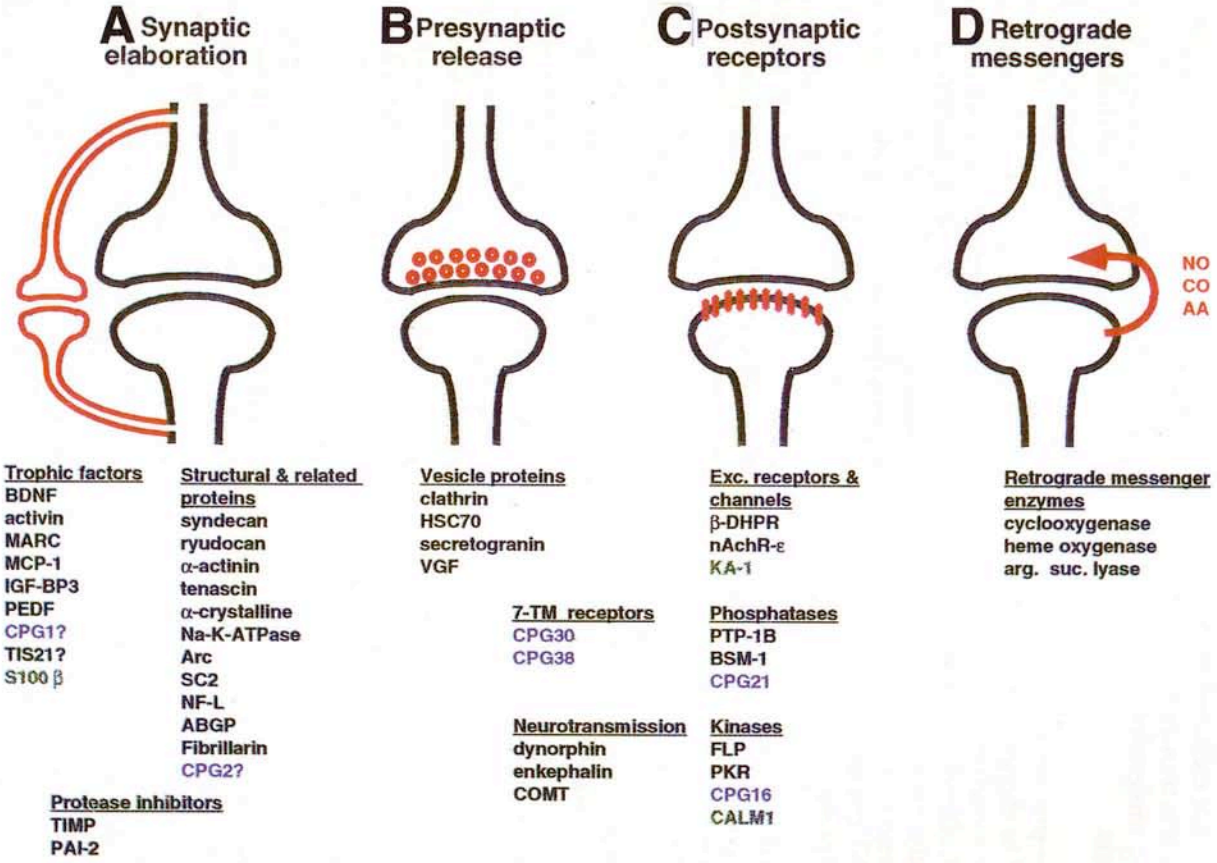

Fig. 6. Models of synaptic plasticity and potential involvement of relevant CPGs. Both CPGs and dCPGs are listed according to their potential functions according to four different models of synaptic plasticity. The cellular changes proposed to underlie synaptic plasticity in each model are highlighted in red. New genes identified through our studies are marked in blue. CPGs that undergo downregulation (dCPGs) are indicated in green. *See* Discussion and Table 2 for details.

all consistent with involvement of a significant fraction of the entire CPG population in neural plasticity.

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GenBank Accession Numbers

The accession numbers described in this article are U78857 (*cpg16*) and U78875 (*cpg20*).

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