Isolation and developmental expression of a rat cDNA encoding a cysteine-rich zinc finger protein

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ABSTRACT

A number of cysteine-rich proteins have recently been isolated by homology screening, differential library screens, and association with other proteins. In this report, we describe the isolation of the rat cysteine-rich protein from a rat brain library during a search for clones with homology to the δ -opioid receptor. One of the cDNAs isolated hybridized to a 1.8 kb mRNA abundantly expressed throughout the rat brain as well as in rat liver. *In situ* hybridization reveals a wide distribution in rat brain; in particular, abundant hybridization was detected in the hippocampus, cerebellum, habenula, reticular thalamic nucleus and interposed nucleus. Nucleotide sequence analysis of a 1403 bp cDNA clone indicated 77% identity with the cDNA for human cysteine-rich protein (hCRP), that translates into a 99% identity at the amino acid level. The predicted amino acid sequence suggests four zinc fingers, two of the C₄ class and two of the C₂HC class. This structural motif is characteristic of members of the LIM domain protein family. The mRNA is serum-inducible in Balb/c 3T3 cells. Additional study suggests that its expression is not induced by either NGF treatment of PC12h pheochromocytoma cells, or inflammation-induced injury in the spinal cord at up to 60 min after injury. It does appear to be developmentally expressed in rat brain, consistent with a potential role in neuronal development. The rat CRP clone will be useful for studying the function of CRPs in rodent models.

INTRODUCTION

Recently, a number of proteins have been found which contain a conserved cysteine-rich domain, CX₂CX_{15–19}HX₂CX₂CX₂CX_{16–21}CX_{2–3}(C,H,D), termed the LIM domain (1, 2). The LIM domain was originally named for the *C.elegans* cell lineage gene *lin-11*, required for the asymmetric division of vulval blast cells, the mammalian insulin gene enhancer binding protein gene *Isl-1*, and the *C.elegans mec-3* protein gene, which is required for the differentiation of a set of touch receptor neurons (1). The LIM domain has subsequently been found in a proto-oncogene,

rhombotin, (3), an adhesion plaque protein, zyxin (4), and the cysteine-rich proteins from human (5), chicken (2), and quail (6). The proposed functions of the LIM domain proteins include the control of gene expression, determination of cell fate and differentiation, control of cell growth and protein–protein interactions at the cytoskeleton.

The cysteine-rich proteins (CRPs) have been isolated by a number of cloning and biochemical techniques as varied as their proposed functions. The human cysteine-rich protein was isolated from a placental cDNA library in a low-stringency search for prolactin transcripts (5). The rat cysteine-rich protein 2 was isolated in a library screen for hepatocyte-like growth factors (7). The chicken cysteine-rich protein was isolated as a zyxin binding protein from avian smooth muscle (2). The quail cysteine-rich protein was isolated as a gene whose expression is suppressed in transformed cells (6). In this report, we describe the isolation of the rat cysteine-rich protein (rCRP) from a rat olfactory bulb cDNA library during a search for clones with homology to the δ -opioid receptor.

In an effort to elucidate the biological function of rCRP, we assessed its expression following treatments shown to induce transcription factors. We examined its expression in PC12h pheochromocytoma cells after treatment with NGF, and in rat spinal cord after inflammation-induced nociceptive, or painful, injury. In addition, we plotted its ontogeny in rat brain to see if it was developmentally expressed.

MATERIALS AND METHODS

Isolation and sequencing of rat cDNA clones

A Sprague–Dawley rat olfactory bulb λ ZAP cDNA library constructed by a combination of random priming and oligo-dT priming was acquired from Stratagene (La Jolla, CA). The library was initially screened with a random primed probe prepared from the coding region of DOR-1 (kindly provided by Dr Chris Evans, UCLA) which was labeled to a specific activity $>5 \times 10^8$ dpm/ μ g (8). The first screen was at moderate stringency, 55°C overnight in 1 M NaCl, 1% SDS, 10% dextran sulfate, 50 mM Tris–HCl, pH 8.0, and 100 μ g/ml denatured salmon sperm DNA. The filters (NEN colony screen plus, Boston, MA) were then washed to a final stringency of $2 \times$ SSC, 1% SDS, 55°C.

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Out of 1×10^6 plaques screened, one positive partial clone was obtained, 14C1. This clone was used to screen the library at higher stringency (60°C hybridization and 65°C washes in $0.1 \times \text{SSC}$, 0.1% SDS) to isolate a clone, 19A1, containing the entire reading frame of DOR-1 (9) as well as a clone, 8A1, with a high degree of sequence homology to the human cysteine-rich protein (5). As 8A1 appeared to be a partial clone, it was then used to screen the library a second time. For the second screen, the filters (NEN colony screen plus) were washed to a final stringency of $0.1 \times \text{SSC}$, 1% SDS, 60°C . After the second screen, seven clones were isolated, two of which were nearly full-length and contained the entire reading frame. cDNA sequences were determined by the dideoxy method (8) and analyzed using the GCG program (Genetics Computer Group, Madison, WI). All sequences were determined on both strands.

Cell culture and serum induction

PC12h cells (kindly provided by Dr Hiroshi Hatanaka, Department of Neurochemistry, Mitsubishi-Kasei Institute of Life Sciences) were grown in media containing 1:1 Ham's F12:DMEM (Dulbecco's modified Eagle's medium) with 10% horse serum. The cells were pretreated for 5 min–10 days with NGF (50 ng/ml , 2.5 S form). NG108-15 neuroblastoma $\times$ glioma hybrid cells (kindly provided by Dr Ping-Yee Law, University of Minnesota) were grown according to standard culture conditions. Other cell lines employed in these studies (Balb/c 3T3, CHO, C6) were purchased from the American Type Culture Collection and cultured under the recommended conditions.

Serum induction of Balb/c 3T3 cells was essentially as described by Wang *et al.* (1992). Briefly, Balb/c 3T3 cells were grown in DMEM supplemented with 10% fetal calf serum (FCS) to 80% confluency and switched to DMEM with 0.5% FCS for 48 h. For the serum induction, the cells were incubated in DMEM with 20% FCS for the indicated times.

Animals

Male Sprague–Dawley rats (250 g , Harlan, Indianapolis, IN), and pups on postnatal days 3–21 (day of birth = day 0) bred in our colony were used in all experiments. To examine the expression of rCRP after an inflammation-induced nociceptive stimulus, animals (18 and 21 days old, and adults) were injured with a subcutaneous injection of a small amount ($5 \mu\text{l}$ for pups, $50 \mu\text{l}$ for adults) of dilute (15%) formalin solution (10). 5–60 min after injury, the animals were euthanized and their lumbar spinal cords rapidly removed. Tissues from unmanipulated control animals were also included for comparison. All whole animal experimental procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

RNA extraction and blot analysis

Total RNA was extracted from cells or tissue homogenates by a modification of the acid phenol extraction technique (11) as previously described (12). The animals ($n = 5-6$) were decapitated, their brains or lumbar spinal cords quickly removed and immediately frozen in liquid nitrogen. The cells or tissues were homogenized in 4 M guanidinium thiocyanate, 25 mM sodium citrate, $\text{pH } 7.0$, 0.5% sarcosyl, 50 mM β -mercaptoethanol. The RNA was extracted with equal volumes of H_2O -saturated phenol, chloroform plus 0.15 M sodium acetate, $\text{pH } 4.5$, and precipitated with an equal volume of

isopropanol. The pellet was washed with 70% ethanol and dried, and finally resuspended in 1 mM EDTA, $\text{pH } 7.0$.

Equal quantities of RNA were denatured by heating in loading buffer for 5 min and loaded onto 1% formaldehyde–agarose horizontal gels as previously described (12). Loading buffer consisted of 50% formamide, 6% formaldehyde, 20 mM boric acid, 10% glycerol, 0.2 mM EDTA, 0.25% bromphenol blue and 0.25% xylene cyanol. After denaturation, $1 \mu\text{l}$ of 1 mg/ml ethidium bromide was added to the sample to permit viewing. This amount of ethidium bromide does not interfere with RNA blot hybridization. The gels consisted of 1% agarose in 20 mM boric acid, $\text{pH } 8.3$, 0.2 mM EDTA, and 3% formaldehyde and were run in a buffer of identical composition. After electrophoresis, gels were soaked for 20 min in 0.05 N NaOH, rinsed in RNase-free water, soaked in $20 \times \text{SSC}$ for 45 min and transferred in $20 \times \text{SSC}$. The gels were transferred to reinforced nitrocellulose by capillary transfer (8), then UV-irradiated to fix the RNA onto the filter.

The probe employed in the RNA blot analysis was the original clone, 8A1, isolated during the library screen. A 1.4 kb insert containing the rCRP sequence was radiolabeled by random priming with T4 DNA polymerase (8). The blots were hybridized in a buffer consisting of 50% formamide, $6 \times \text{SSC}$, 0.1 mg/ml salmon sperm DNA, 50 mM Tris, $\text{pH } 8.0$, and $5 \times \text{Denhardt's}$ ($50 \times \text{Denhardt's}$ is 1 g Ficoll, 1 g polyvinylpyrrolidone, 1 g BSA fraction V in 100 ml H_2O). Filters were then hybridized at 42°C using 10^7 dpm of radiolabeled probe (specific activity $> 5 \times 10^8 \text{ dpm}/\mu\text{g}$). The filters were washed to a stringency of $0.5 \times \text{SSC}$, 0.1% SDS, 60°C .

To control for differences in loading, a second oligonucleotide probe was prepared from 28S rRNA ($5'$ AAA ACG ATG AGA GTA GTG GTA TTT CAC CG, complementary to bp 3762–3790 of rat 28S rRNA) by end-labeling with T7 polynucleotide kinase and used to re-probe the blots for comparison. Since many housekeeping genes, like actin, are developmentally expressed (13), or, like GAPDH, dependent on cell cycle (14), 28S rRNA was deemed the most appropriate control (15). Before re-probing, the filters were slowly boiled in $0.1 \times \text{SSC}$, 0.1% SDS for 20 min. Because of the lower T_m of the oligonucleotide probe, these blots were washed at 30°C .

The washed blots were exposed to film for 24 h or longer. The hybridization signal was quantified by two separate means. Blots were apposed to film and the resulting autoradiograms were illuminated on a light box and digitized with a solid-state video camera coupled to an imaging system designed for quantitative densitometry (Microcomputer Imaging Device, Imaging Research Inc., St Catharines, Ontario). The relative optical density units (ROD) were used for comparison. Alternatively, the blots were counted using a Molecular Dynamics PhosphorImager (Molecular Dynamics, Sunnyvale, CA). This is now our method of choice for quantitation, as the results are linear over a greater range than with film, although all films quantitated in this study were within the linear range.

The normalized optical densities were analyzed using one way analysis of variance with postnatal age as the factor. The Student–Newman–Keuls *post hoc* analysis was performed when appropriate.

In situ hybridization

The protocol previously described by Young (16) was employed for *in situ* hybridization. Brains were rapidly removed and quickly

frozen in Tissue-Tek O.C.T. compound (Miles Inc., Elkhart, IN). The tissue was then cut (12 μ m), thaw-mounted onto gelatin-coated slides, and stored at -20°C . Before hybridization, the sections were thawed on aluminum foil for 10 min. They were then post-fixed with 4% paraformaldehyde in PBS, pH 7.4, rinsed twice in PBS, and treated for 10 min with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl, pH 8.0. The sections were dehydrated in increasing concentrations of ethanol, delipidated

in chloroform, transferred through decreasing concentrations of ethanol, and air dried. The sections were hybridized in a buffer which consisted of 50% formamide, 600 mM NaCl, 4 mM EDTA, 0.1% sodium pyrophosphate, 0.2% SDS, 0.2 mg/ml sodium heparin, 10% dextran sulfate, 80 mM Tris-HCl, pH 7.5. Hybridization was performed at room temperature using $1-2 \times 10^6$ dpm of ^{32}P -radiolabeled probe per section (specific activity was $> 5 \times 10^8$ dpm/ μ g). The probe employed in these

Figure 1. (A) Nucleotide and protein sequences of the rat cysteine-rich protein (rCRP). The putative zinc finger domains as well as the associated glycine-rich regions have been underlined. The GenBank accession number for rCRP is U09567. (B) A comparison of rCRP with human CRP (hCRP) (5), chicken CRP (cCRP) (2), quail CRP (qCRP) (6), rat CRP2 (rCRP2) (7) and CRIP (23). Conserved amino acid residues among 5 or more of the proteins are shaded. The LIM domains are indicated. Gaps in the sequence alignment are indicated by dots (.) and the end of the sequences are indicated by asterisks (*).

studies was an oligonucleotide complementary to bp 342–302 of rCRP (5' TTG GTG GTA GGC CTG TGT CCG GGG GCT TCC TCA TGC TTG AT). Competition with unlabeled oligonucleotide was used as a control (17, 18). 1 μ g unlabeled oligonucleotide was added to half of the sections, washed off and then labeled oligonucleotide was added for the hybridization. The sections were washed to a final stringency of $2\times$ SSC, 25% formamide, 30°C. Slides were again dehydrated through increasing concentrations of ethanol, air dried and apposed to film.

RESULTS

Isolation of rat cysteine-rich cDNA

The initial objective of the present study was to identify and characterize transcripts that were related to the δ -opioid receptor. A Sprague–Dawley rat olfactory bulb λ ZAP cDNA library, which was prepared by a combination of random priming and oligo-dT priming, was obtained from Stratagene. The library was screened at moderate stringency with a random primed probe prepared from the coding region of DOR-1 (19). Out of 1×10^6 plaques screened, two positive clones (19A1 and 8A1) were analyzed further. Analysis of these clones revealed that 19A1 was a rat homolog of the δ -opioid receptor (9).

In addition to the rat δ -opioid receptor, clone 8A1 was analyzed. Clone 8A1 had a high degree of sequence homology (87% in the first 127 bp sequenced) with the human cysteine-rich cDNA (5). Because 8A1 appeared to be missing the first 60 bp of the coding region of the human cysteine-rich cDNA, it was used to re-screen the library. Seven more partial clones were detected after a second screen, two of which were nearly full-length and contained the entire reading frame. One of the

two longer clones (16DA) was sequenced and employed for the following studies. This clone has 37% identity overall at the nucleic acid level with the δ -opioid receptor, and was probably isolated in the initial library screen due to its abundance in the library and the high GC content of both sequences.

Analysis of the rCRP cDNA sequence

Sequence analysis of the clone 16DA isolated from the rat olfactory bulb cDNA library revealed it to be a rat homolog of the human cysteine-rich protein (hCRP). The nucleotide and putative protein sequence of the rat cysteine-rich protein (rCRP) cDNA are shown in Figure 1A. The translation initiation codon was defined by analogy with the hCRP sequence. The rat sequence is 77% identical to the hCRP at the nucleic acid level, while the predicted amino acid sequence shows 99% identity (5). The four putative zinc finger domains as well as the associated glycine-rich region have been underlined, and again have been defined based on the hCRP sequence. Sequence analysis shows similarity with other cloned cysteine-rich proteins (Fig. 1B). In particular, the chicken (2) (73.4% and 90% identity at the nucleic and amino acid levels, respectively) and quail cysteine-rich proteins (6) (74% and 80% identity at the nucleic and amino acid levels, respectively) are highly conserved. The overall homology with the rat cysteine-rich protein 2 (rCRP2) is lower (7). Sequence analysis with rat CRP and rCRP2 reveals 45% identity at the nucleic acid level and 53% similar, 38% identical amino acids. Although CRP has 37% amino acid identity with rCRP, the zinc finger and glycine-rich domains are highly conserved.

Distribution and expression in rat and cultured cells

RNA blot analysis of total RNA extracted from rat tissues suggests an abundant distribution of rCRP (Fig. 2). The ~ 1.8 kb message was located in rat liver and all brain areas examined (hippocampus, striatum, pituitary, hypothalamus, brainstem, cerebellum, midbrain and cortex). The transcript was also found in a number of cell lines, including C6 glioma, CHO, NG108-15 neuroblastoma $\times$ glioma hybrid cells, Balb/c 3T3 and PC12h pheochromocytoma cells (data not shown).

Using hCRP as a probe, serum induction of CRP expression had previously been demonstrated in rat fibroblasts and Balb/c 3T3 cells, suggesting a role for CRP as a primary response gene

Figure 2. The tissue distribution of rCRP mRNA in adult rats. Each lane contains equivalent amounts of RNA (10 μ g). The band corresponding to the rCRP mRNA (rCRP) is indicated with an arrow. The RNA molecular weight markers are noted on the left. This blot was apposed to film for 2 weeks.

Figure 3. Serum induction of CRP expression in Balb/c 3T3 cells. Serum-starved cells were exposed to 20% fetal calf serum for the indicated times. Each lane contains 10 μ g of RNA. The bands corresponding to the rCRP mRNA (rCRP) and to 28S rRNA (28S) are indicated with arrows. Dashes denote the RNA molecular weight markers. This figure represents a 1 day exposure.

(20). Similarly, using rCRP as a probe, serum induction was observed with Balb/c 3T3 cells (Fig. 3). Another system that has been shown to induce the expression of several immediate early genes is NGF stimulation of pheochromocytoma cells. However, the expression of rCRP is not induced by NGF treatment of PC12h cells. No change in expression is observed following either short- or long-term treatment (data not shown).

In situ hybridization indicates widespread distribution in brain, thereby confirming the results of the RNA blot analysis. Abundant hybridization to the rCRP probe was detected in the hippocampus, cerebellum, habenula, reticular thalamic nucleus and interposed nucleus (Fig. 4A). The signal obtained with the rCRP probe could be blocked by competition with 1 μ g cold probe (Fig. 4B).

Developmental expression and expression after injury

As can be seen in Figure 5, rCRP mRNA is present as early as postnatal day 3 in the rat brain, and appears to steadily increase with age. The relative abundance of the message in the 3 day old animals (normalized to 28S rRNA) was less than half that

observed in the adult animals (Figs 5 and 6). An analysis of variance performed on the normalized optical densities indicates a significant increase in the expression of rCRP in whole brain associated with increasing age ($F[4,20] = 10.61$, $P < 0.001$). The 3, 10 and 15 day old pups expressed significantly less rCRP mRNA than the adults ($P < 0.05$). In addition, rCRP mRNA levels in the 3 and 10 day old pups were also significantly lower than in the 21 day old pups ($P < 0.05$).

The levels of rCRP expression are not induced after inflammation-induced nociceptive stimuli (data not shown). Injury did not appear to alter expression at any of the post-injury times assessed. An analysis of variance performed on the data confirmed that no significant differences between the post-injury groups were present ($P > 0.05$, data not shown).

DISCUSSION

During a library screen for cDNAs related to the δ -opioid receptor, we identified a cDNA clone which a sequence search

Figure 4. Distribution of rCRP in rat brain. This figure represents a horizontal section of adult rat brain hybridized with an oligonucleotide probe directed against bp 302–342 of rCRP (A). The signal obtained with the rCRP probe could be blocked with 1 μ g cold probe (B).

revealed to be homologous with the human cysteine-rich protein. Subsequent analyses indicated relatively little sequence similarity between rCRP and the δ -opioid receptor at the nucleic acid level. The clone has 37% identity overall at the nucleic acid level with the δ -opioid receptor, and was probably isolated in the initial library screen due to its abundance in the library and the high GC content of both sequences. The predicted amino acid sequence and function appear to be equally dissimilar (38% similarity, 15% identity). It is interesting to note that Liebhaver and colleagues (5) found the human cDNA (hCRP) during a low stringency screen for transcripts similar to the human prolactin gene (hPrI), a gene with an equally dissimilar predicted protein product and function.

Both the nucleic and amino acid sequences of the isolated clone (16DA) had high sequence homology with members of the CRP family. The rat sequence demonstrates 77% identity to the hCRP at the nucleic acid level, while the predicted amino acid sequence shows 99% identity. The differences in the protein sequence can be accounted for by two amino acids, one of which is a conserved change (Thr93 to Met, Ala153 to Ser, respectively). The avian CRPs are also highly identical. For example, the chicken has 73.4% and 90% identity at the nucleic and amino acid levels, respectively, and the quail cysteine-rich proteins have 74% and 80% identity at the nucleic and amino acid levels, respectively (2, 6). The first glycine domain also overlaps with a potential nuclear localization signal, KKYGPK (21, 22). Chicken CRP has been shown to be associated with the cytoskeleton (2), although nuclear localization for a CRP has not yet been demonstrated.

Abundant hybridization to the rCRP probe was detected in a number of rat tissues, including several brain regions and liver. The CRP mRNA had not been detected previously in liver (20). This apparent difference is probably due to the longer exposure

time employed in the present study (2 weeks). However, we can detect a signal in the liver upon overnight exposure. It may be that upon longer exposure, the CRP2 mRNA is detected, which has been demonstrated to be present in liver (7). Alternatively, there is only 77% overall identity between rCRP and hCRP, so rCRP should be a more sensitive probe in rat tissues.

To determine the distribution of CRP mRNA in the brain, *in situ* hybridization was performed. Hybridization was observed in the hippocampus, cerebellum, habenula, reticular thalamic nucleus and interposed nucleus. These are all areas, with the possible exception of the reticular thalamic nucleus and the interposed nucleus, with high cell density which generally display intense staining. Therefore, although specific hybridization to the rCRP probe has been demonstrated, these results may indicate that rCRP is present in all neural tissue. Hybridization was not observed in fiber tracts or white matter. These data are suggestive of a distribution in neurons, however, they do not preclude the possibility that rCRP may be expressed in glia in grey matter.

A number of LIM domain-containing proteins, including CRIP (23), are developmentally expressed. In addition, cCRP levels in the gizzard have recently been demonstrated to increase during embryogenesis (2). Our data indicate that rCRP mRNA is present as early as postnatal day 3 in the rat, and that it steadily increases in expression with increasing postnatal age. This report documents the first example of a member of the CRP family with developmental expression in the brain. Because rCRP is so abundant in the cerebellum, the developmental increases reported in the present study may actually be a reflection of cerebellar proliferation and ontogeny. We have previously demonstrated that cannabinoid receptor mRNA expression in the cerebellum increases postnatally; paralleling the postnatal ontogeny of the underlying tissues (12). It is important to note, however, that cerebellar development is relatively complete by postnatal day 10 (24), while rCRP continues to display significant increases in expression (Fig. 6). Therefore, the developmental regulation observed in the present study probably represents more than simple development of the underlying tissue.

Figure 5. Comparison of rCRP mRNA expression by RNA blot hybridization in rat pups of varying postnatal ages and adults. Each lane contains equal amounts of RNA (10 μ g). The bands corresponding to the rCRP mRNA (rCRP) and to 28S rRNA (28S) are indicated with arrows. Dashes denote the RNA molecular weight markers. This figure represents an 8 day exposure of the blot.

Figure 6. Expression of rCRP mRNA in whole brain, normalized to 28S rRNA and expressed in mean ($\pm$ SEM) optical density units. $n = 5$ samples at each point. The rCRP mRNA levels in 3, 10 and 15 day old pups were significantly lower than in the adults ($P < 0.05$, indicated by single asterisks, *), while the levels in 3 and 10 day old pups were also significantly lower than in the 21 day old pups ($P < 0.05$, indicated by double asterisks, **).

The rat CRP, like the other CRPs, contains two copies of the LIM motif followed by a glycine-rich domain. Both the domains are highly conserved within the CRP/CRP2/CRIP proteins. Their conservation throughout evolution and abundant tissue distribution suggests that CRPs may be essential to cell function. Evans and Hollenberg (25) have suggested several possible advantages favoring zinc finger transcription factors. However, there is no direct evidence that LIM proteins lacking homeodomains are transcription factors. In *mec-3* (26) and *isl-1* (27), the LIM domains appear to inhibit the binding of the homeodomain to DNA. Although the structure of the CRP C-terminal LIM domain shows a structural similarity to GATA-1 and steroid hormone receptor DNA binding domains (28), compelling evidence that LIM domains interact with DNA is lacking.

The function of the CRPs is not yet understood. The human CRP has been shown to be a primary response gene that is induced in quiescent fibroblasts in response to serum stimulation in parallel with *c-myc* (20). Studies with the quail CRP reveal that its 0.9 kb mRNA is present in normal quail and chicken embryo fibroblasts, but is undetectable in cells transformed by retroviruses. These results, and the relationships of the CRPs to the LIM homeodomain-containing proteins, suggest DNA binding and the regulation of cell growth as a possible function. On the other hand, the chicken CRP has been shown to associate with another LIM domain-containing protein, zyxin, that is associated with the actin cytoskeleton (2). However, these data may not be so disparate. Crawford *et al.* have observed that another cytoskeletal protein, merlin, may function as a tumor suppressor in humans (2, 29). Our data are consistent with both these proposed roles, as we find it in normal and transformed cells.

It has been shown that several transcription factors, including c-Fos, c-Jun and KROX-24 [aka zif/268, NGFI-A, Egr-1 (30)] are expressed in the lumbar spinal cord in response to inflammation-induced nociceptive stimuli (for review see 31). Therefore, to elucidate a possible function for rCRP, we examined its expression in spinal cord after inflammation-induced injury, a nociceptive stimulus previously shown to induce KROX-24. No significant differences were found between the post-injury groups. The times examined, up to 1 h, correspond to the peak nociceptive response (10, 32) and other data with c-Fos. It is possible, however, that any changes in expression of rCRP may have occurred later.

In summary, the rat cysteine-rich protein is highly homologous to the human CRP, with 99% amino acid identity. The availability of a rat CRP clone will facilitate the study of the function of CRP in rats. Finally, the results from the present study indicate that rCRP may be developmentally regulated in neural tissues. Therefore, elucidation of the function of rCRP may illuminate new facets of neuronal development.

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