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Mouse C-reactive protein

Generation of cDNA clones, structural analysis, and induction of mRNA during inflammation

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A full-length C-reactive protein (CRP) cDNA clone has been isolated from a CBA/J-strain-mouse acute-phase liver library. The 1614-nucleotide cDNA specifies mRNA 5′ and 3′ untranslated regions of 81 and 858 bases respectively that flank 675 bases encoding mouse pre-CRP. The derived amino acid sequence predicts a 19-residue leader peptide followed by a 206-residue mature mouse CRP that shows considerable sequence identity with both human and rabbit CRP. Northern-blot analysis of mouse liver CRP mRNA concentrations after inflammatory stimuli and comparison with hepatic induction of mRNA for the major mouse acute-phase protein serum amyloid P component established that CRP, a major acute-phase reactant in human and rabbit, is a minor acute-phase reactant in mouse. The size and organization of the mouse CRP mRNA 5′ and 3′ untranslated regions are significantly different from those of human and rabbit CRP mRNA and may have implications for its anomalous minimal induction during acute inflammation.

INTRODUCTION

The pentraxins are a family of serum proteins, members of which have been recognized in a range of evolutionarily diverse species from *Limulus* (horseshoe crab) to higher mammals (reviewed in [1]). Pentraxins have a native pentameric configuration of five non-covalently bound identical subunits organized as a planar disc. In mammals there are two pentraxins: C-reactive protein (CRP) and serum amyloid P component (SAP); the former is found as a single disc [2], the latter as two discs arranged face-to-face [3]. CRP and SAP are synthesized, principally in liver, as pre-molecules from which typical hydrophobic leader sequences are cleaved [4,5] to yield mature monomers of 206 [6] and 204 [5] amino acids respectively. Their sequences are highly conserved [6] and, in man, the genes for both map to band q2.1 of chromosome 1 [7]. In mouse, the SAP gene maps to a region of the distal portion of mouse chromosome 1 [8,9] which is syntenic with the region of human chromosome 1 containing band 12.1 (reviewed in [10]).

During the mammalian acute-phase response to inflammation one of the most dramatic among a spectrum of physiological changes is the increase in serum concentration of a number of proteins called 'acute-phase reactants' (reviewed in [1]). In man and rabbit, one of the serum proteins that exhibits the highest proportional increase, from trace resting levels to several hundred micrograms per ml, is CRP. SAP is a relatively low-concentration constitutive serum protein in man. Paradoxically, in mouse it is SAP that is a 'major' acute-phase reactant, increasing from resting levels of around 60 μg/ml or less to peak concentrations of several hundred micrograms/ml during acute inflammation [11–13]. Although mouse CRP is poorly defined, it has been reported as a trace serum component [14] that increases slightly in concentration during the acute-phase response [11,15].

In this paper we characterize a full-length CRP complementary DNA clone isolated from a CBA/J strain mouse acute-phase liver library. The CRP mRNA specified differs significantly from the human and rabbit CRP mRNAs; the derived mouse CRP amino acid sequence is compared with those of human and rabbit CRP. In addition, a modest hepatic induction of mouse CRP mRNA following inflammatory stimuli is described and contrasted with the much more emphatic induction of SAP mRNA. The implications of the structural and synthetic anomalies of mouse CRP are discussed.

MATERIALS AND METHODS

Mouse cDNA synthesis and library construction

A CBA/J strain mouse acute-phase liver cDNA library was constructed essentially by the method of Caput *et al.* [16]. Briefly, 250 ng of polyadenylated RNA was purified from acute-phase total liver RNA (see below) using oligo(dT)–cellulose (Pharmacia, Piscataway, NJ, U.S.A.) chromatography [17]. cDNA was synthesized by avian-myeloblastoma-virus reverse transcriptase (Life Sciences, St. Petersburg, FL, U.S.A.) using a synthetic oligonucleotide, 5′-GATCCGGGCCC$(T)_{12}$-3′, as primer. Residual RNA was removed by alkaline hydrolysis. The cDNA was (dC)-tailed and sized by Sepharose CL4B column chromatography before hybridization with the adaptor 5′-$(A)_{12}$GGGCCCG-3′ and with plasmid vector PTZ19 (Pharmacia) that had

Abbreviations used: CRP, C-reactive-protein; SAP, serum amyloid P component; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

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These sequence data have been submitted to the EMBL/GenBank Data Libraries.

been linearized with restriction endonuclease *Pst*1, (dG)-tailed, further digested with *Bam*H1, and sized by Sepharose CL4B column chromatography. Subsequent ligation, and repair of the single-stranded region with T4 polymerase, produced recombinant molecules in which the inserts were bounded at their 5′ termini by oligo(G) preceded by a *Pst*1 site and at their 3′ termini by a *Bam*H1 site reconstituted by the 'sticky' ends of the primer/adaptor and vector. These were used to transform competent *Escherichia coli* MC1061 host cells, giving 1.5×10^5 ampicillin-resistant recombinant bacteria.

Isolation of mouse CRP cDNA clones

Recombinants (30000 in all) were screened on duplicate nitrocellulose filters by a modification of the method of Grunstein & Hogness [18]. Two 'generic' CRP oligonucleotides (see below) were 5′-labelled using [γ-^{32}P]ATP (Amersham, Arlington Heights, IL, U.S.A.) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA, U.S.A.) and hybridized to filter-bound recombinant DNA for 16 h at 40 °C in 6 × SSC/1 × Denhardt's solution/0.05% sodium pyrophosphate/tRNA (100 μg/ml). Filters were subsequently washed with 0.9 M-NaCl/90 mM-sodium citrate at 50 °C for 15 min. Three duplicate hybridization signals were revealed by autoradiography. The colony giving the strongest signal (MRCP1) was grown in bulk and recombinant plasmid was purified by the cleared-lysate method [19].

Sequence analysis

The insert of MCRP1 was directly sequenced using oligonucleotide primers and a Sequenase kit (U.S. Biochemical Corp., Cleveland, OH, U.S.A.) via the dideoxy-chain-termination method of Sanger *et al.* [20], as modified by the manufacturer for sequencing from double-stranded templates.

Computer analysis

Nucleotide and amino acid sequence analyses were performed using PCGENE software (Intelligenetics, Mountain View, CA, U.S.A.), alone, and in combination with the Bionet Resource (National Institutes of Health grant P41RR01685). Sequence comparisons facilitated by the above were by the method of Pearson & Lipman [21].

Mouse CBA/J strain acute-phase liver RNA samples

The RNA samples used in the present study were generated to permit mRNA levels for a range of major and minor mouse acute-phase proteins to be monitored after inflammatory stimuli [13]. In brief, female CBA/J mice were given a single intraperitoneal injection of 1 ml of 3% (w/v) thioglycollate or a single subcutaneous injection of 0.1 ml of 10% azocasein. At timed intervals (1, 2, 4, 8, 12, 18, 24, 36, 48 and 72 h) three animals were killed by cervical dislocation and livers were removed and frozen immediately in liquid N_2 before RNA extraction by the LiCl/urea method [22]. Livers from unstimulated control animals killed at 0, 36 and 72 h were processed similarly to permit prestimulation levels of test mRNA species to be determined and to ensure that deviations from prestimulation levels were due to experimentally induced inflammation and not to changes in the animal-care environment.

Northern-blot analysis

RNA samples (20 μg) were size-fractionated by electrophoresis on 1.2% agarose/formaldehyde gels and transferred to nitrocellulose filters (Schleicher and Schuell, Keene, NH, U.S.A.). Filters were washed in 2 × SSC, dried, and baked for 2 h at 80 °C. Baked filters were prehybridized at 45 °C in 6 × SSC/5 × Denhardt's/0.5% SDS/0.05% sodium pyrophosphate/salmon sperm DNA (100 μg/ml) for 4 h. CRP- and SAP-specific oligonucleotides (100 ng) were 5′-labelled with T4 polynucleotide kinase (Amersham) and [γ-^{32}P]ATP (Amersham) for use as hybridization probes. Unincorporated [γ-^{32}P]ATP was removed by passing over prepacked Sephadex G-25 columns (NAP-10; Pharmacia) and the specific radioactivities of the labelled oligonucleotides determined. Hybridization was overnight under the conditions defined above. Filters were subsequently washed in 6 × SSC/0.1% SDS/0.05% pyrophosphate at 60 °C for 30 min; signals were revealed by autoradiography.

Oligonucleotides

All oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer.

For screening the cDNA library, two 'generic' CRP oligonucleotide probes were constructed. The sequences were chosen according to the following rationale. The amino acid sequences of human [6,23] and rabbit [24] CRP are identical for residues 28–37 (KPLKAFTVCL) and 153–163 (VGDIGNVNMWD); furthermore, the nucleotide sequences encoding these regions are identical except for a single base difference in the first case. Therefore, two 'generic' CRP oligonucleotides, GECRP2 (5′-GAGGCACACAGTGAAGGCTTTGAGTGGCTT-3′) and GECRP1 (5′-GTCCCACATGTTCACATTTC-CAATGTCTCCCAC-3′), which are complementary to the rabbit mRNA encoding the above regions, were synthesized.

A total of fourteen 18-base-long oligonucleotides were synthesized for use as sequencing primers after initial confirmation of the specificity of clone MCRP1 from sequence obtained using GECRP1 and GECRP2 as primers.

For Northern-blot analysis of liver RNA, a SAP oligonucleotide (5-ATTAGGATTGACAGGGGAAT-CTCTGTACAC-3′) complementary to nucleotides 730–749 of the mouse SAP mRNA sequence reported by Ishikawa *et al.* [25] and a CRP oligonucleotide (5′-CTTGTGCAGACTTTTCCGCACCTTGGGTTT-3′) complementary to nucleotides 478–507 of the mouse CRP mRNA described in the present study were synthesized. These 30-nucleotide-long probes each contained 50% GC/50% AT to allow the use of identical hybridization and wash conditions. Both oligonucleotides were sized by passing them over NAP-10 columns (Pharmacia). Their concentrations were determined by A_{260} before labelling.

RESULTS AND DISCUSSION

The CBA/J-strain mouse is an excellent model for the study of the acute-phase response to inflammation *in vivo*. After an inflammatory stimulus, hepatic synthesis of mRNA for the complement components factor B, C3 and C5 is modestly induced and hepatic synthesis of mRNA for the major acute-phase proteins SAP and

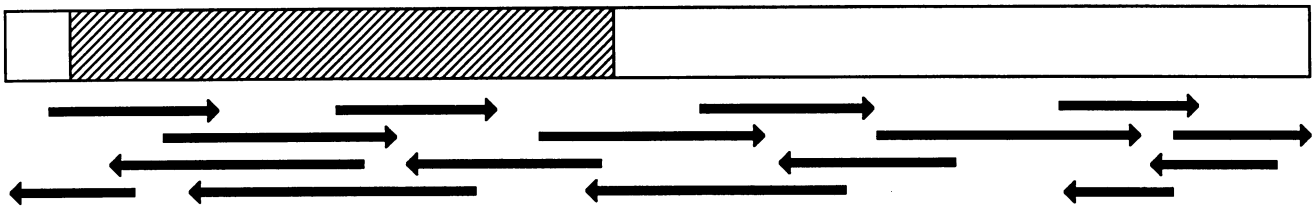

Fig. 1. Strategy for sequencing clone MCRP1

Diagrammatic representation of clone MCRP1 encompassing the 5′-untranslated region (open box on left), sequence encoding mouse pre-CRP (hatched box) and the 3′-untranslated region (open box on right). A total of eight specific oligonucleotides were used to sequence 93.5% of the sense strand and eight specific oligonucleotides were used to sequence 92.3% of the anti-sense strand.

serum amyloid A component is dramatically induced [13]. In addition, SAP complementary [9] and genomic [26] DNA clones have been isolated from CBA/J strain libraries and characterized. The CBA/J strain was therefore chosen for our molecular-genetic studies of mouse CRP and our investigation of the acute-phase induction of mouse CRP mRNA.

Generation of a mouse acute-phase liver cDNA library

Polyadenylated RNA was isolated from total RNA extracted from the liver of a CBA/J mouse 4 h after intraperitoneal injection of thioglycollate. Complementary DNA was synthesized and inserted into the PTZ19 plasmid vector by a method which yields a high proportion of recombinant molecules bearing full-length cDNA. Transformation of the *E. coli* host strain MC1061 with the above gave approx. 1.5×10^5 independent ampicillin-resistant bacteria.

Isolation and sequencing of a mouse CRP cDNA clone

Preliminary experiments indicated that human CRP cDNA [6] does not cross-hybridize with mouse CRP sequences. The amino acid sequences of human [6] and rabbit [24] CRP contain several regions of identity encompassing nine or more residues. For some, the conservation at the protein level reflects a similar degree of conservation in mRNA sequences. Two such regions, which differ markedly from the corresponding portions of SAP, were selected, and 'generic' oligonucleotides capable of hybridizing to either human or rabbit CRP mRNA were synthesized. Assuming that they would also hybridize to mouse CRP cDNA, we used the oligonucleotides as probes to screen 30000 colonies of the mouse acute-phase liver cDNA library. The most prominent positive colony, MCRP1, was isolated.

The above strategy was successful despite several mismatches between each oligonucleotide and the mouse CRP cDNA, which were revealed by subsequent sequence analysis of MCRP1. Therefore, in cases where existing cDNA clones do not cross-hybridize, oligonucleotide probes based on regions of high sequence identity may be used to identify complementary and genomic pentraxin clones from other mammalian species.

Nucleotide sequence of mouse CRP cDNA

The cDNA insert of MCRP1 was sequenced on both strands using specific oligonucleotide primers as outlined in Fig. 1. MCRP1 contains 1614 nucleotides comprising 756 nucleotides encoding pre-CRP flanked by 81 nucleotides specifying mRNA 5′-untranslated region and the entire 858 nucleotides of mRNA 3′-untranslated region (Fig. 2).

The first 39 nucleotides of MCRP1 are more than 75% identical with nucleotides 2–40 and 4–42 of the human [23] and rabbit [24] CRP mRNAs respectively (results not shown). Therefore MCRP1 is full length or within a few bases of full length. The 5′-untranslated region of mouse CRP mRNA (at approx. 81 nucleotides) is thus somewhat shorter than those of the human (104 nucleotides) and rabbit (113 nucleotides) CRP mRNAs. The 15 nucleotides closest to the ATG codon specifying the *N*-terminal methionine residue of mouse pre-CRP are similar to those found in the corresponding regions of the human and rabbit CRP mRNAs (results not shown). Between the flanking portions of the mouse CRP mRNA 5′-untranslated region defined above are 27 nucleotides that are fewer in number and different in sequence from those found in the analogous portions of the human and rabbit CRP mRNAs (results not shown). The mouse CRP mRNA 5′-untranslated sequence contains two possible heat-shock-protein consensus (CTnGAAnnTTCnAG) sequences [27]: CcaGgAcTcCttG between nucleotides 8 and 20, and tTGAtctTTCAG between nucleotides 24 and 35. Such consensus sequences are located at equivalent positions in the human and rabbit CRP mRNAs. If previous speculations [10] that these elements are involved in the control of CRP-gene expression are valid, then the considerable differences in spatial organization and sequence of the mouse CRP mRNA 5′-untranslated region relative to the high-identity human and rabbit CRP mRNA 5′-untranslated regions may account, in part, for the low acute-phase induction of mouse CRP mRNA defined below.

The 3′-untranslated region, at 858 nucleotides, is considerably shorter than the 3′-untranslated regions of the human (approx. 1.3 kb) and rabbit (approx. 1.5 kb) CRP mRNAs. A long 3′-untranslated region (of similar size to those of human and rabbit CRP mRNA) is required for maximum transcriptional induction of *Drosophila* heat-shock protein 26 RNA in response to elevated temperature [29]. As inflammation is generally associated with fever, it has been suggested [10] that the long 3′-untranslated region of human CRP mRNA contributes to the optimum acute-phase induction of CRP transcription and possibly mRNA stability. If so, the much shorter 3′-untranslated region of mouse CRP mRNA might account, at least in part, for the minimal acute-phase induction observed below.

The 3′-untranslated region reported by us differs in two places from that recently reported by Ohnishi *et al.*

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   1 AGGCGTTCCAGGACTCCTTGTCCTTGATCTTTCAGACAAAACACTGTCCTCTTAGTCCAG
  61 ATCCCAGCAGCATCCATAGCCATGGAGAAGCTACTCTGGTGCCTTCTGATCATGATCAGC
 121 TTCTCTCGGACTTTTGGTCATGAAGACATGTTTAAAAAGGCCTTTGTATTTCCCAAGGAG
 181 TCAGATACTTCCTATGTGTCTCTGGAAGCAGAGTCAAAGAAGCCACTGAACACCTTTACT
 241 GTGTGTCTCCATTTCTACACTGCTCTGAGCACAGTGCGCAGCTTCAGTGTCTTCTCTTAT
 301 GCTACCAAGAAGAACTCTAACGACATTCTCATATTTTGGAATAAGGATAAACAGTATACT
 361 TTTGGAGTGGGTGGTGCTGAAGTACGATTCATGGTTTCAGAGATTCCTGAGGCTCCAACA
 421 CACATCTGTGCCAGCTGGGAGTCTGCTACGGGGATTGTAGAGTTCTGGATTGATGGGAAA
 481 CCCAAGGTGCGGAAAAGTCTGCACAAGGGCTACACTGTGGGGCCAGATGCAAGCATCATC
 541 TTGGGGCAGGAGCAGGACTCGTATGGCGGTGACTTTGATGCAAAGCAGTCTTTGGTGGGA
 601 GACATCGGAGATGTGAACATGTGGGATTTTTGTGCTATCTCCAGAACAGATCAACACAGTC
 661 TATGTTGGTGGGACACTCAGCCCCAATGTTTTGAACTGGCGGGCACTGAACTATAAAGCA
 721 CAGGGTGATGTGTTTATTAAGCCGCAGCTGTGGTCCTGACCTACTGTTGTGAACCCTGAA
 781 GCACCTCCTGGGATTACATTCTCTCCCTTGTCTCGGGTTATGAACCTTTTAGCCCCAGCA
 841 GATGTTGTAGGTCTGTTCTGTGAATATGGCCTTTCACTTCTCTGCTTTGTGGTCCTCAGC
 901 ACTAGAGCACGGAATTTAAATGGAAGGCTTCCAGCATAAGCATCCCACTAGGACTCTACC
 961 AAAGAGAATCTGACTTACCCATGGTTTTATATATATATGTAAATATCCATATATATATAT
1021 ATATGCATATATATATATATATAATTGAAAAAATTTCAGACATAATTCTTCTCCCTCACA
1081 TAGATGAGAAAATAGATGCACAGAAAGGAGAATAATTTTTTATTGTTTTTGTTTTATAAT
1141 GTCATCTTGAGTGCTGTATTTACATACTTTCTATCCCTCCCTCTTCAGATCCTTTCCTAT
1201 CCTTCCAAATTCTCTCTCAAATTCATGATGTCTTATTATTAGTCTTATGCATATATACAT
1261 ATGCATAATACCTATCATCTATCAATCAATCTATCTACCTATCTATCATCTATTCATCAG
1321 TCATCCATCTTACTGATTACATTTAGTGCTTCTTGTATTTTGTTGAAGACTGGACACTGG
1381 ATAATCTATCAGGAGGGCCCCTCCCTGAAGACTGATTGTCCTTTTCTCAGCAGCCACTGA
1441 TTACCTCTAGCTCTTCATATAGGGTTCTGTCTTTGTGAAATTTCTTCTGTCCATGTTGCA
1501 TGTCAATTGGTGTCATTATGCAGGTCTTGTTTGGGCAACCTAGAGTGATGGAGCACTGAC
1561 TACACTGTGCTCAGAATCAGTTCTTTTCTGGAATAAAATCTGTACCTGAACTTC
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Fig. 2. Nucleotide sequence of clone MCRP1

The 1614 nucleotides were determined by the sequencing strategy outlined in Fig. 1. The sequence encoding pre-CRP is enclosed by halved square brackets. The ATG and TGA triplets specifying the *N*-terminal methionine and stop codon respectively are underlined. Also underlined are the putative heat-shock consensus elements [27] between nucleotides 8 and 20 and between 24 and 35, the extensive AT repeat region between nucleotides 989–1045 and the AATAAA polyadenylation signal sequence between nucleotides 1592 and 1597. The following differences between this sequence and that reported by Ohnishi *et al.* [28] are noted: a, at nucleotide 481 Ohnishi *et al.* [28] report a G rather than C; b, immediately after nucleotide 1026 Ohnishi *et al.* reported 30 nucleotides ($AC[AT]_7ACATAC[AT]_4$) that are not present in our sequence: the seven nucleotides ending at position 1026 are CATATAT according to Ohnishi *et al.* [28] rather than TATATGC; c, at nucleotide 1516 Ohnishi *et al.* [28] reported a C rather than a T. The boundaries of the sequences to which the oligonucleotides GECRP1 (nucleotides 595–627) and GECRP2 (nucleotides 220–249) hybridized are indicated (^); mismatches between the target sequences and the oligonucleotides are indicated (▪).

[28]. At position 1516 we observe a T rather than a C, probably reflecting a trivial allelic difference. A major difference is observed between nucleotides 989 and 1045. This region contains an extensive series of AT repeats. The sequence described by Ohnishi *et al.* [28] contains an additional AC(AT)$_7$ACATAC(AT)$_4$ between nucleotides 1026 and 1027. Although this region can, in theory, participate in the formation of stem and loop structures, we do not believe our clone to be the result of reverse transcriptase 'reading across' a looped segment of the mRNA template: the sequence of the seven nucleotides ending in nucleotide 1026 is TATATGC in our clone, whereas the equivalent heptanucleotide in the sequence of Ohnishi *et al.* [28] is CATATAT, suggesting that the differences define significant polymorphic variants. Purine–pyrimidine repeats, such as the multiple AT motifs between nucleotides 989 and 1027, are capable, in theory, of adopting the Z-DNA conformation. There are GT repeat regions in the introns of the human [6,23] and rabbit [24] CRP genes, though there is no such sequence in the intron of the mouse CRP gene reported by Ohnishi *et al.* [28]. If these regions have some role in CRP-gene expression, perhaps as targets for Z-DNA binding proteins similar to those that mediate the co-ordinated expression of simian-virus-40 genes [30], then the major differences in the AT repeat region discussed above might determine differential acute-phase expression of CRP in different mouse strains.

Derived amino acid sequence of mouse CRP

The amino acid sequence of mouse pre-CRP (Fig. 3) specified by MCRP1 is identical with that derived from the mouse CRP genomic sequence published by Ohnishi *et al.* [28], except for a single residue. At position 105 our sequence specifies a proline residue (encoded by a CCC triplet) rather than an alanine (encoded by a GCC triplet). At the equivalent position in human [6,23] and rabbit [24] CRP and in human [5], mouse [25,26,32] and Syrian hamster [31] SAP a proline residue is found. Computer-generated tertiary-structure analysis, using the 'Novotny' program (PCGENE, Intelligenetics), predicts that the proline residue at position 105 of mouse CRP facilitates a turn centred around the aspartate residue at position 102 within the tetrapeptide WIDG (results not shown). Similar analyses of all of the other mammalian pentraxins support the probability of a turn specified by the analogous tetrapeptide preceding the proline residue present in the sequence of each. Computer analysis of the mouse CRP sequence, in which alanine has been substituted for proline 105, predicts a much reduced probability of a turn involving the tetrapeptide WIDG. Therefore, if the proline/alanine difference represents allelic variation, rather than sequencing error, it is likely to have consequences for the tertiary structure of the mature CRP molecules.

In contrast with the division by Ohnishi *et al.* [28] of

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           -19                  1                            38
Mouse      MEKLLWClLimiSFSrtfghedMfKKAFVFPKESDtSYVSLeAesKKPLntFTVCLh
Rabbit     MEKLLWCFLtLvSFSnmsdQagMhKKAFVFPKESDnSYVSLnAqLKKPLKAFTVCLy
Human      MEKLL CFLvLtSlShafgQtdMsrKAFVFPKESDtSYVSLkApLtKPLKAFTVCLh

Conserved  MEKLLwCfL-l-SfS--fgq-dM-kKAFVFPKESDtSYVSL-A-lkKPLkaFTVCLh

                                                             95
Mouse      FYTaLStvRsfSvFSYATkknsNdILiFWnKDkqYtFgVGGaEvrFmVSeIPeaPTH
Rabbit     FYTdLSmTRGYSIFSYATrRQfNEILlFWSKDIGYSFsVGGdEIiFkVSdIPVdPTH
Human      FYTeLSsTRGYSIFSYATkRQdNEILiFWSKDIGYSFtVGGsEIlFeVpevtVaPvH

Conserved  FYT-LS-tRgySiFSYATkrq-NeILiFWsKDigYsF-VGG-Ei-F-VseipvaPtH

                              *                              152
Mouse      iCASWESaTGIvEfWiDGKPkVRKSLhKGYtvGPdASIILGQeQDSyGGdFdakQSL
Rabbit     lCASWESsTGIaElWVDGKPmVRKSLKKGYilGPEASIILGQdQDSFGGsFEkqQSL
Human      iCtSWESasGIvEfWVDGKPrVRKSLKKGYtvGaEASIILGQeQDSFGGnFEgsQSL

Conserved  iCaSWESatGIvEfWvDGKP-VRKSLkKGYtvGpeASIILGQeQDSfGG-Fe--QSL

                                                             206
Mouse      VGDIGdVNMWDfvLSPEqINTvYvGGTlSPNVLnWRaLnYkaqGdVfiKPQLWs
Rabbit     VGDIGNVNMWDyaLSPEEINTIYaGGTFSPNVLdWReLtYqVrGEVhvKPQLWP
Human      VGDIGNVNMWDfvLSPdEINTIYlGGpFSPNVLnWRaLkYeVqGEVftKPQLWP

Conserved  VGDIGnVNMWDfvLSPeeINTiY-GGtfSPNVLnWRaL-Y-vqGeVf-KPQLWp
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Fig. 3. Alignment of the derived mouse, rabbit and human pre-CRP amino acid sequences

Residues conserved between sequences are in upper-case letters; lower case letters indicate residues that are not found in all three CRPs, and in the portion of the Figure detailing conserved sequence indicate residues that are the same in only two of the three CRPs. *, The proline at position 105 differs from the alanine specified by the mouse CRP gene sequence reported by Ohnishi *et al.* [28]. Residues conserved between all mammalian pentraxins reported thus far, i.e. mouse CRP, human CRP [6,23], rabbit CRP [24], Syrian-hamster SAP [31], human SAP [5] and mouse SAP [25,26,32] are underlined. The alignment and determination of residues conserved in all mammalian pentraxins was obtained using the GENALIGN program (PCGENE, Intelligenetics) accessed via the Bionet Resource.

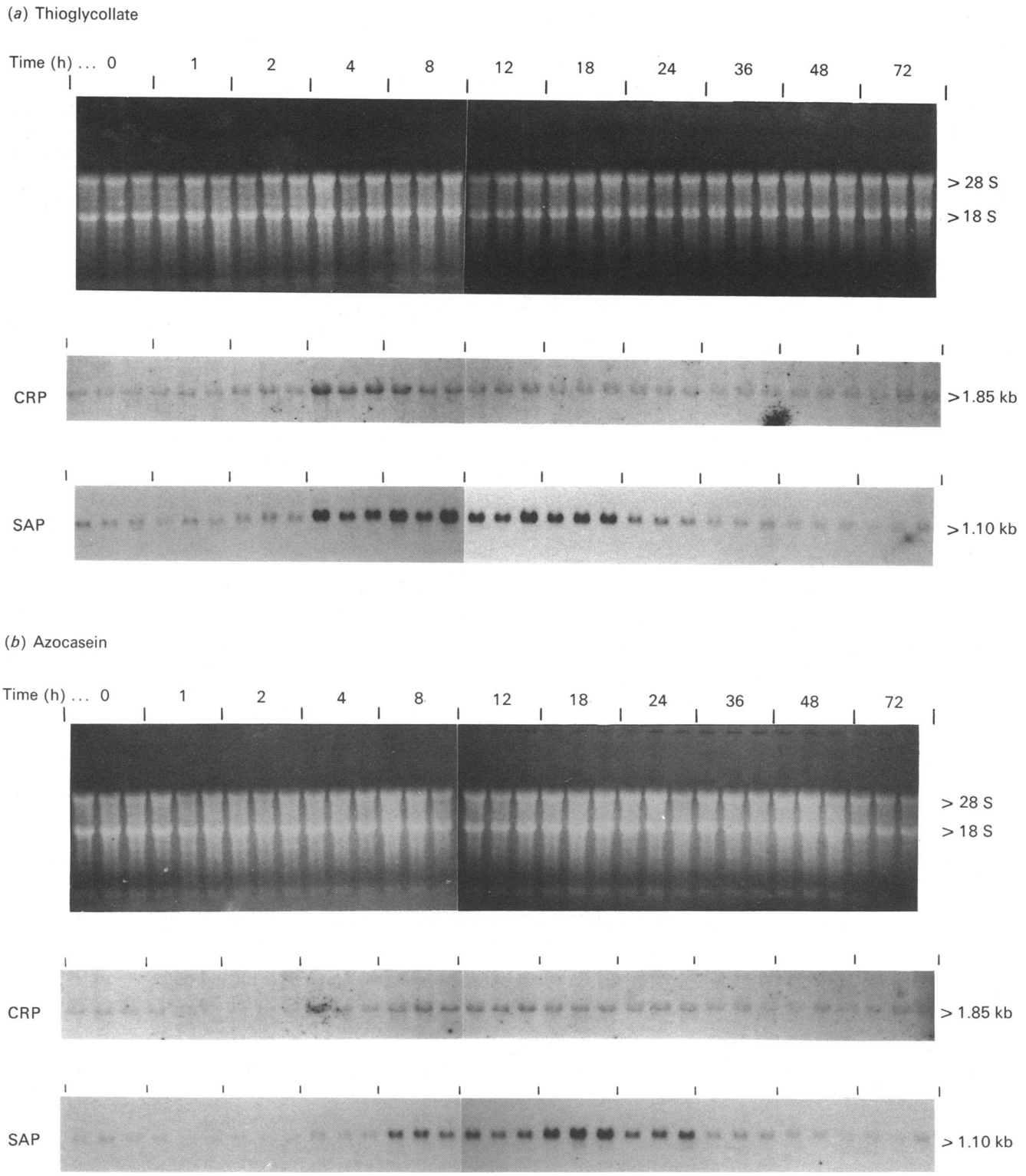

Fig. 4. Analysis of post-stimulation hepatic pentraxin mRNA levels

Analysis of hepatic pentraxin mRNA levels after stimulation by intraperitoneal injection of thioglycollate (*a*) and subcutaneous injection of azocasein (*b*). The upper panels are photographs of the agarose/formaldehyde gels bearing size-fractionated RNA samples (20 μg/track) revealed by u.v. illumination after staining with ethidium bromide. The time points (in h) corresponding to each triplicate set of RNA samples are indicated. The lower two panels are autoradiographs of RNA blots of the same gels probed with a radiolabelled CRP oligonucleotide (middle panels) and a radiolabelled SAP oligonucleotide (lower panels). The CRP and SAP probes were labelled to specific radioactivities of 2.01×10^6 d.p.m./ng and 2.24×10^6 d.p.m./ng respectively; the autoradiographs were produced by exposure at -70 °C in the presence of two intensifying screens for 120 h for the CRP panel and 14 h for the SAP panel. RNA size standards (Pharmacia) were co-run (not shown) to allow size determination of the CRP and SAP mRNAs.

mouse pre-CRP into a leader sequence of 20 residues and a mature polypeptide of 205 residues, we favour the existence of a 19-residue leader sequence and a 206-residue mature molecule, as observed for human CRP. The latter view is supported both by computer analysis using the PSIGNAL program (PCGENE, Intelligenetics), which predicts cleavage by signal peptidases between the glycine residue at position 19 and the histidine residue at position 20 (results not shown), and by the alignment of the derived mouse pre-CRP sequence with the *N*-terminal sequences of mature human CRP [33] and SAP [34], each of which have been directly determined by protein sequencing.

The amino acid sequences of the mouse, rabbit and human pre-CRP molecules are aligned in Fig. 3. There are 127 residues (62%) that are conserved between the mature CRP molecules of the three species. Paired comparisons revealed 138 (67%) identities between mouse and rabbit, 147 (71%) identities between mouse and human, and 156 (76%) identities between rabbit and human. In addition, there are 69 residues, also depicted in Fig. 3, that are invariant between all mammalian pentraxin protein sequences thus far described.

Induction of CRP mRNA by acute inflammatory stimuli

In CBA/J mice, intraperitoneal injection of thioglycollate and subcutaneous injection of azocasein both potently stimulate the hepatic production of acute-phase protein mRNAs, the former giving rise to a more rapid induction characterized by significantly higher peak mRNA levels [13].

To determine whether mouse CRP mRNA is induced during acute inflammation, we analysed Northern blots of total RNA extracted from the livers of triplicate CBA/J mice killed at timed intervals after stimulation with either thioglycollate or azocasein. Fig. 4 depicts the levels of CRP and SAP mRNA detected in the thioglycollate-induced samples (Fig. 4*a*) and the azocasein-induced samples (Fig. 4*b*). For each, a photograph of the gels (upper panels) was taken before RNA transfer to nitrocellulose filters; these confirm that similar quantities of each RNA sample were loaded and subsequently analysed by hybridization of the resulting Northern blots with radiolabelled CRP (middle panels) and SAP (lower panels) oligonucleotides. Peak SAP mRNA levels occur at 8–12 h after thioglycollate injection, whereas peak levels after azocasein injection are at 18 h. SAP mRNA levels begin to rise 2 h after the former stimulus and 4 h after the latter; the accumulation of SAP mRNA in response to thioglycollate injection is quantitatively greater. These results agree with those previously observed by us [13]. In response to thioglycollate injection, CRP mRNA, like SAP mRNA, is elevated by 2 h; however, the peak levels are observed earlier, at 4 h. Azocasein injection also induces elevated levels of CRP mRNA first observed, like SAP mRNA, at 4 h; again the peak CRP mRNA concentrations are observed earlier, at 8–12 h. The induction of CRP mRNA by thioglycollate injection is quantitatively greater.

The profiles in Fig. 4 establish that CRP mRNA concentrations in mouse liver rise after an inflammatory stimulus. The CRP and SAP mRNA profiles were produced by using oligonucleotide probes of similar specific radioactivities; however, the CRP panels required 120 h autoradiography, whereas the SAP panels required only 14 h. Although strict quantification of CRP and SAP mRNA levels has not been performed, we estimate that peak CRP mRNA concentrations are approx. 20-fold lower than peak SAP mRNA concentrations. Mouse CRP is therefore a minor acute-phase reactant. Interestingly, Ciliberto *et al.* [35] have shown that human CRP mRNA can be dramatically induced in the livers of transgenic mice bearing a human CRP gene, indicating that the mouse has the signal-transduction apparatus necessary for greatly increased production of CRP mRNA. Resolution of this apparent paradox will require further analysis of the control of expression of both human and mouse CRP and the detailed characterization of the promoter and enhancer elements present in the CRP genes of each species.

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