

Human Serum Amyloid P Component

cDNA ISOLATION, COMPLETE SEQUENCE OF PRE-SERUM AMYLOID P COMPONENT, AND LOCALIZATION OF THE GENE TO CHROMOSOME 1*

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Complementary DNA clones corresponding to the human serum amyloid P component (SAP) were isolated, and the complete nucleotide and derived amino acid sequence of preSAP was determined. PreSAP biosynthesis is directed by a 1.1-kilobase mRNA. Synthesis and postsynthetic processing of preSAP in *Xenopus* oocytes result in secretion of a protein with mobility similar to native purified SAP when analyzed by sodium dodecyl sulfate gel electrophoresis. The human SAP gene is on chromosome 1, probably closely linked to the gene for C-reactive protein which encodes the related acute phase reactant found in human plasma.

The pentraxins, a family of proteins found in the sera of many species, share primary amino acid sequence homology and display a characteristic discoid arrangement of five non-covalently bound subunits (1, 2). Human serum contains two pentraxins: serum amyloid P component (SAP¹), a precursor of the protein amyloid P component found in nearly all amyloid deposits (3, 4), and C-reactive protein (CRP). Although these two proteins are similar, they are distinguished by (a) ligand binding specificity, (b) the fact that SAP is glycosylated and CRP is not, (c) SAP is decameric (two pentameric discs face to face) and CRP is pentameric, and (d) SAP serum concentrations are relatively constant (20–40 µg/ml) while CRP concentrations rise from normal (1–2 µg/ml) to more than 100 µg/ml during an acute phase response (reviewed in Ref. 5).

Recently, cDNA clones corresponding to human CRP have been isolated and used to localize the CRP gene to human chromosome 1 (6), as well as to analyze the molecular regulation of CRP gene expression (7). In the present report, we describe the isolation of human SAP-specific cDNA clones, provide the nucleotide and derived amino acid sequences for the SAP primary translation product, and compare the biosynthesis and postsynthetic processing of human SAP and CRP. In addition, evidence is presented that the SAP gene is also on chromosome 1, probably closely linked to CRP, pro-

viding further support for the hypothesis that these are products of a gene duplication event.

MATERIALS AND METHODS

Preparation of Synthetic Oligonucleotides—A mixture of 256 different 20-nucleotide long oligonucleotides, containing all sequences that could code for the amino acids of human SAP between residues 102 and 108 (residue number according to Ref. (8)), was synthesized by a solid phase phosphotriester method using a library of dimer anions (9). In addition, three unique sequence 18-mer oligonucleotides were synthesized by a modified phosphotriester method on a Biosearch Sam One automated nucleotide synthesizer, for use as primers during nucleotide sequencing.

Isolation of SAP cDNA Clones—An adult human liver cDNA library containing more than 200,000 recombinant clones (10) was screened using a modification of the Grunstein-Hogness method (11). The library was plated at high density on 82-mm nitrocellulose filters and probed with the oligonucleotide mixture, 5' end-labeled with adenosine [γ -³²P]triphosphate (New England Nuclear) and T4 polynucleotide kinase (Bethesda Research Laboratories) at a concentration of 50 ng/ml in 0.9 M NaCl, 90 mM Na citrate, 1 × Denhardt's solution, 0.05% Na pyrophosphate, 100 µg/ml tRNA (12). Filters were washed repeatedly with 0.9 M NaCl, 90 mM Na citrate, 0.05% Na pyrophosphate at 20 °C and once at 42 °C for 10 min in the same buffer. Positive hybridization signals were visualized by autoradiography.

Plasmids were prepared by the alkaline lysis method (13), and the SAP-specific cDNA inserts were isolated by agarose gel electrophoresis after restriction endonuclease digestion with *Pst*I (New England Biolabs, Beverly, MA). Nucleotide sequencing of SAP cDNA was accomplished by the method of Maxam and Gilbert (14) and by the dideoxynucleotide chain termination method using inserts subcloned into the M13 vector mp11 (15).

Messenger RNA Isolation and Analysis—Human hepatic RNA was obtained as previously described (10) from a cadaver organ transplant donor, and the polyadenylated poly(A⁺) fraction was purified by affinity chromatography on oligo(dT)-cellulose (16). Poly(A⁺) mRNA was size fractionated by agarose-formaldehyde gel electrophoresis and transferred to nitrocellulose filters (17, 18). Following nick translation (19), the 613-base pair (bp) *Pst*I fragment of SAP cDNA clone (pSAP1) was used as a hybridization probe. SAP mRNA was visualized by autoradiography on Kodak XAR-5 film following prehybridization, hybridization, and washing using standard conditions (20).

RNA Translation—The mRNA was translated in the presence of [³⁵S]methionine (500 µCi/ml) (New England Nuclear) in a rabbit reticulocyte lysate system (21) and in *Xenopus* oocytes (22). Oocytes were each injected with 50 µg of poly(A⁺) hepatic RNA and incubated at 20 °C in Barth's medium (10 µl/oocyte) containing [³⁵S]methionine (500 µCi/ml) in microtiter wells each containing four oocytes. Media from separate wells were pooled, 48 h after injection, and the oocytes from wells containing four to eight oocytes in a total volume of 40–80 µl were pooled, washed with phosphate-buffered saline, and homogenized in lysis buffer (phosphate-buffered saline containing 1% Triton X-100, 0.5% Na deoxycholate, 10 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride, pH 7.6). The homogenate was centrifuged at 12,000 × g for 5 min at 4 °C, after which an equal volume of

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¹ The abbreviations used are: SAP, serum amyloid P component; CRP, C-reactive protein; kb, kilobase; bp, base pairs.

1% sodium dodecyl sulfate in phosphate-buffered saline was added. The culture media were mixed with an equal volume of lysis buffer containing sodium dodecyl sulfate.

Immunoprecipitation and Analysis of Translation Products—Reticulocyte lysate translation products were diluted 6-fold with 0.5 × lysis buffer with 5 mM EDTA and incubated with 4 µl of antiserum to human SAP (obtained from Atlantic Antibodies, Scarborough, ME) at 4 °C overnight. Oocyte lysate and culture media were analyzed using the same antiserum. Fifty microliters of a 10% suspension of protein A (IgG Sorb, Enzyme Center, Cambridge, MA) in lysis buffer with 5% bovine serum albumin was added to each sample and incubated at 0 °C for 1 h. Complexes were sedimented by centrifugation at 12,000 × *g* for 1 min, washed in lysis buffer, solubilized by boiling in Laemmli sample buffer (23), and analyzed by sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis. The radiolabeled products were visualized by autoradiography.

Purified human CRP and SAP isolated using previously described techniques (24) were electrophoresed in parallel with immunoprecipitates of translation products and visualized after staining with Coomassie Blue.

Cell Lines—The somatic cell hybrids were derived from fusions of the hypoxanthine-guanine phosphoribosyltransferase-deficient Chinese hamster cell E36 or mouse cell RAG with white blood cells or fibroblasts from three unrelated individuals. Two of the white blood cell donors were female carriers of reciprocal X/19 translocation chromosomes: the X/19W translocation t (X;19) (q23-25;q13) (25) and the X/19B translocation t (X;19) (q1;p13) (26). The fibroblast donor was a karyotypically normal male. The hybrid clones were characterized for human chromosome complements by both isozyme and cytogenetic techniques (27). In addition, the DNAs used in the present study were analyzed with cloned DNA probes for 21 of the human autosomes and the X chromosome.

Isolation and Analysis of Human, Rodent, and Somatic Cell Hybrid DNA—High molecular weight DNA from human peripheral blood lymphocytes, mouse RAG cells, hamster E36 cells, and somatic cell hybrids was prepared (28). After digestion with various restriction endonucleases, the DNA was size-fractionated by agarose gel electrophoresis, alkali-denatured, and then transferred to nitrocellulose filters (29). The resulting blots were hybridized under standard conditions (30) with the large *Pst*I fragment of pSAP1, which had been radiolabeled by nick translation. Filters were washed in 30 mM NaCl, 3 mM Na citrate at 65 °C for 1 h. Hybridization signals were visualized by autoradiography.

RESULTS

Isolation and Sequence Analysis of SAP cDNA—The oligonucleotide mixture (Fig. 1) was used to screen 25,000 recombinant colonies in duplicate. Sixteen positive clones were identified of which two (pSAP1 and pSAP11) were selected for further analysis. Fig. 2 shows the relative sizes of these two clones and the sequencing strategy employed. Clone pSAP1 contains 734 bp, extending from the codon specifying amino acid residue 30 for SAP through the stop codon followed by 150 bp of the 3' untranslated region and a 20-bp poly(A) tail. Clone pSAP11 is 919 bp in length and contains 38 bp of the 5' untranslated region, the codons for a 19 amino acid signal peptide, the 204 amino acid residues of the mature SAP protein, the 3' untranslated region, and a poly(A) tail. The complete nucleotide sequence of preSAP and the 3' untranslated region is shown in Fig. 3.

	102						108
Amino Acid	Gln	Gly	Tyr	Phe	Val	Glu	Ala
mRNA	5' CA ^A _G	GG ^U _C	UA ^U _C	UU ^U _C	GU ^U _C	GA ^A _G	GC ^U _A 3'
Synthetic Oligonucleotides	3' GT ^T _C	CC ^A _T	AT ^A _G	AA ^A _G	CA ^A _T	CT ^T _C	CG 5'

FIG. 1. Composition of the synthetic oligonucleotide mixture corresponding to amino acid residues 102–108 (8) used as hybridization probe to screen the adult human liver cDNA library.

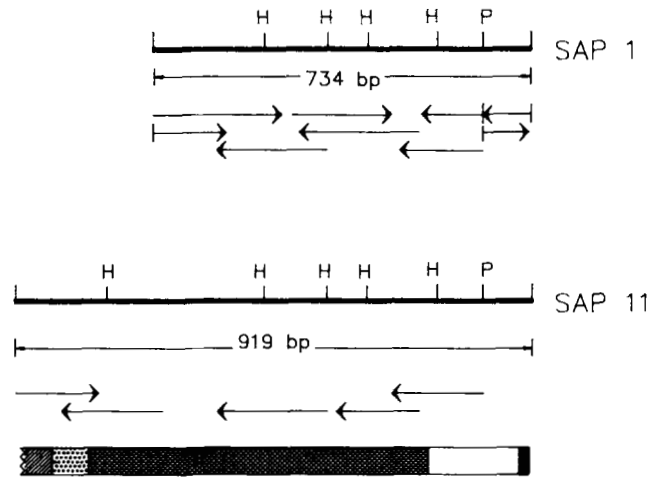


FIG. 2. Size comparison and nucleotide sequencing strategy for pSAP1 and pSAP11. Restriction endonuclease recognition sites are designated P, (*Pst*I) and H (*Hinf*I). Arrows start at priming sites and indicate the length of sequence generated. Arrows beginning with bars indicate fragments sequenced by the method of Maxam and Gilbert (14) and others by the method of Sanger *et al.* (15). The lower panel shows a schematic representation of the organization of the nucleotide sequence for pSAP11 (striped bar, 5' untranslated region; dotted bar, signal peptide region; hatched bar, coding sequence; open bar, 3' untranslated region; solid bar, poly(A) tail). Sequence was generated for 64% of the coding strand and 84% of the complementary strand of pSAP1 and 63% of the anti-coding strand and 9% of the coding strand of pSAP11. Overlapping sequence was determined for the entire coding region of SAP at least two times.

Biosynthesis and Postsynthetic Processing of SAP—A 1.1-kilobase (kb) SAP-specific mRNA species was identified in RNA isolated from human liver when Northern blots were probed with the large *Pst*I (613 bp) fragment of pSAP1 (Fig. 4a). Hybridization of the same filter with a human CRP-specific cDNA probe (pCRP5) revealed a 2.2-kb mRNA species (data not shown) in agreement with a previous study (7). Under the conditions of this experiment, no hybridization was detected between SAP mRNA and pCRP5 or CRP mRNA and pSAP1.

Under cell-free conditions, human hepatic RNA directed synthesis of a single chain protein (apparent *M_r* ~ 26,000) precipitable with antiserum to human SAP (Fig. 4b, lane 1). A diffuse radiolabeled band (Fig. 4b, lane 2) (apparent *M_r* ~ 28,000) with an electrophoretic mobility similar to native purified SAP (Fig. 4b, lane 4) was detected in lysates of *Xenopus* oocytes injected with human hepatic mRNA. Proteins with similar mobility (three distinct bands) (Fig. 4b, lane 3) were found in the oocyte extracellular medium after immunoprecipitation with an aliquot of the same antiserum to SAP. Lysates and media from oocytes injected with water served as controls, and no SAP-specific bands were detected (data not shown).

Chromosomal Localization of the Human SAP Gene—Somatic cell hybrids between human leukocytes and mouse RAG or hamster E36 cells (6, 25–27), which carried a full complement of rodent chromosomes and a limited but varied human chromosome complement, were analyzed for the presence of the human SAP gene. DNA was isolated from the peripheral blood lymphocytes of eight unrelated individuals, digested with the restriction endonuclease *Bam*HI (New England Biolabs), and subjected to Southern blot analysis, using the radiolabeled pSAP1 as a hybridization probe. A fragment of approximately 4.8 kb was detected for each individual (data not shown). DNA isolated from somatic cell hybrid lines was

FIG. 3. Nucleotide sequence for SAP cDNA (pSAP1 and pSAP11). The derived amino acid sequence for SAP is compared with the amino acid sequences for human CRP (Ref. 33 and P. Woo and A. S. Whitehead, personal communications) and the Syrian hamster female protein (32). Gaps have been introduced in the depiction of SAP, CRP, and female protein (*FP*) sequences to maximize homology. The numbering system refers to SAP.

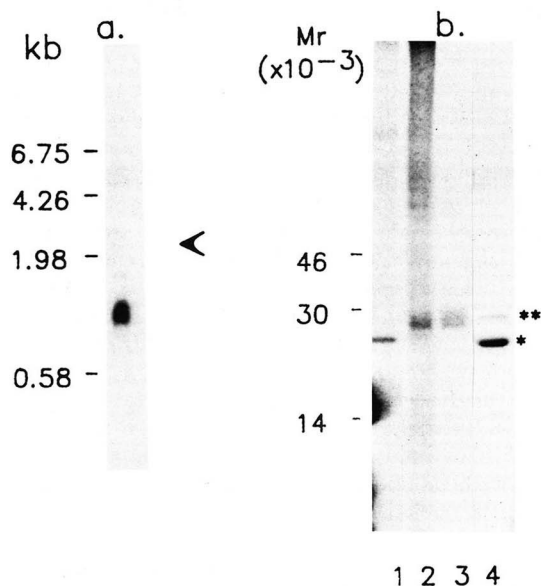
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FIG. 4. *a*, Northern blot analysis of SAP-specific RNA. The arrow-head designates the relative position of migration of CRP-specific mRNA. *b*, immunoprecipitation of translation products. Lane 1, SAP-specific immunoprecipitate from cell-free translation in rabbit reticulocyte lysate system; lane 2, SAP immunoprecipitate from cell lysate of *Xenopus* oocytes injected with human liver RNA; lane 3, SAP immunoprecipitate from culture media of *Xenopus* oocytes injected with human liver RNA; lane 4, relative electrophoretic mobility of purified native human SAP (**) and CRP (*).

subjected to similar analysis and assessed for the presence or absence of the human 4.8-kb band (Fig. 5). The presence or absence of this band is concordant only with the presence or absence of human chromosome 1 (Table I) permitting assignment of the SAP gene to this chromosome.

Linkage Analysis of SAP and CRP—In a preliminary study, high molecular weight DNA from peripheral blood lymphocytes of two unrelated individuals was digested with six dif-

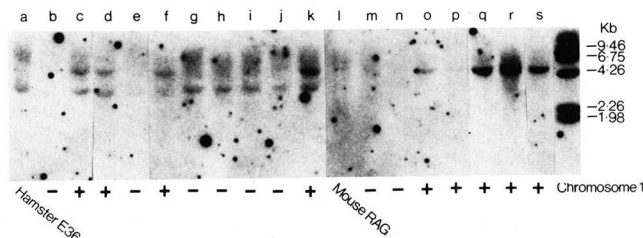


FIG. 5. Southern blot hybridization of pSAP1 with *Bam*HI-digested DNA from hamster-human and mouse-human somatic cell hybrids and rodent parent cell lines. The DNA samples in each track are: *a*, hamster parent cell line E36; *b-k*, hamster-human hybrids G35-C2, -D5, -B4, -B5, -A4, -C5, -E3, -F1, -F3, and -D2; *l*, mouse parent cell line RAG; mouse-human hybrids: *m*, G24-A9; *n*, G17-15; *o*, RRP 5-7; *p*, G24-B7; *q*, RRP 3-6; *r*, RRP 5-3; *s*, G24-B5. The 4.8-kb band represents human SAP; the 3.4-kb band is visualized in all hamster and hamster-human cell lines. The presence or absence of chromosome 1 is shown *below* each track.

ferent restriction endonucleases (*Hind*III, *Pst*I, *Pvu*II, *Bam*HI, *Xba*I, and *Kpn*I) (New England Biolabs), each with hexanucleotide recognition specificity. The DNA was subjected to Southern blot analysis in duplicate and probed with pSAP1 and pCRP5. Restriction fragments generated by digestion with *Hind*III, *Pst*I, *Pvu*II, and *Bam*HI and fragment lengths were specific for each probe (data not shown). In contrast, digests with *Xba*I and *Kpn*I generated fragments of 5.3 and 6.0 kb, respectively, to which both pSAP1 and pCRP5 hybridized (Fig. 6). To determine whether fragments with the same or similar mobility were detected by the two probes, the positive signal generated with the radiolabeled SAP cDNA was allowed to decay. The blot was then hybridized with the CRP probe. The resulting 5.3- and 6.0-kb bands were indistinguishable from the bands detected in the original autoradiograph.

DISCUSSION

In this report, we describe the isolation and nucleotide sequence of cDNA clones corresponding to human serum

TABLE I

Correlation of human chromosome content with the presence of the SAP gene

Data are summarized from hybridization of pSAP1 to DNA from somatic cell hybrids (Fig. 5). Concordant: ++ indicates presence of the 4.8-kb human SAP-specific band and presence of the chromosome; -- indicates the absence of both. Discordant: +- indicates presence of the 4.8-kb band and absence of the chromosome; -+ indicates absence of the 4.8-kb band and presence of the chromosome.

Human chromosome	Hybridization of probe				Total	
	Concordant		Discordant		Concordant	Discordant
	++	--	+-	-+		
1	9	8	0	0	17	0
2 ^a	2	6	6	2	8	8
3 ^a	6	5	3	2	11	5
4	6	5	3	3	11	6
5	4	4	5	4	8	9
6	5	6	4	2	11	6
7	5	5	4	3	10	7
8	2	2	7	6	4	13
9	2	6	7	2	8	9
10	7	3	2	5	10	7
11 ^a	6	4	3	3	10	6
12 ^a	7	2	2	4	9	6
13 ^a	3	4	5	3	7	8
14	5	4	4	4	9	8
15	5	6	4	2	11	6
16	5	4	4	4	9	8
17 ^a	4	4	5	3	8	8
18 ^a	7	3	2	4	10	6
19 ^{a,b}	6	0	1	7	6	8
20	5	2	4	6	7	10
21	5	3	4	5	8	9
22	5	6	4	2	11	6
X ^{a,c}	6	6	3	1	12	4
Y	1	8	8	0	9	8

^a Clones with a chromosome present in less than 15% of metaphases or in which a chromosome-specific isozyme or DNA probe exhibited only a weak signal were excluded from analysis for that chromosome. Clones with a rearranged chromosome were likewise excluded from the analysis.

^b The chromosome 19 line includes the intact 19 and the 2 different 19q⁺ translocation chromosomes.

^c The X line represents the intact X and the 2 Xq⁻ translocation chromosomes.

amyloid P component. The complete nucleotide sequence for the SAP primary translation product was generated from two SAP-specific clones. The larger of these clones (pSAP11) contains a 919-bp SAP-specific insert but does not span the entire SAP mRNA (estimated size, 1.1 kb) since it lacks a portion of the 5' untranslated region. This clone was used to determine the nucleotide and derived amino acid sequence of the signal peptide for preSAP. This sequence for preSAP is similar to the sequences of signal peptides for other secreted proteins (31).

The amino acid sequence of native SAP derived from the nucleotide sequence is similar to the primary sequence of SAP protein reported by Anderson and Mole (8), but differs in several respects. The most important of these differences is noted in the region between residues 65 and 98 (number according to Ref. 8), corresponding to residues 65 and 116 in the sequence reported here. The cDNA sequence defines a realignment of the peptide specified by Anderson and Mole between residues 67 and 80 (67-99 in the derived sequence) and two regions (78-85 and 100-116) not identified at the protein level. We believe the cDNA sequence represents the authentic SAP sequence and is not a result of a cloning

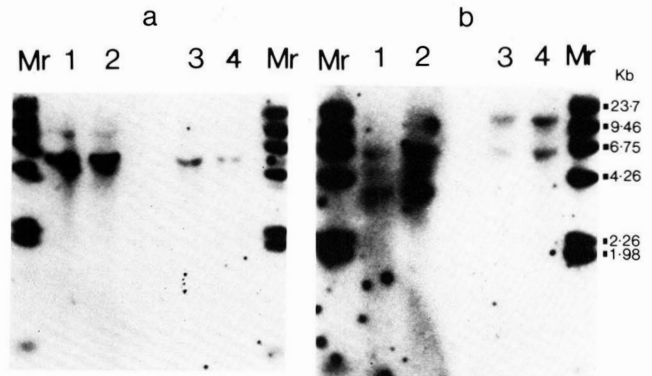


FIG. 6. Southern blot analysis of human DNA from peripheral blood cells probed with pCRP5 (lanes 1 and 2) and pSAP1 (lanes 3 and 4). DNA samples (15 µg/slot) from two unrelated individuals digested with *Xba*I (panel a) and *Kpn*I (panel b) are shown, as well as DNA from one individual in lanes 1 and 2 and the other in lanes 3 and 4. Filters were cut following transfer hybridized with the respective cDNA probes and rematched or autoradiography. *Hind*III-digested λ phage served as size markers in flanking lanes. Common bands 5.3 and 6.0 kb, respectively, for *Xba*I and *Kpn*I digests were visualized when probed with pCRP5 and pSAP1.

artifact for several reasons: (a) two SAP cDNA clones demonstrate identity of structure based on restriction mapping and partial nucleotide sequencing of pSAP11 and complete sequence analysis of pSAP1; (b) the SAP cDNA sequence aligns with the sequence of a related pentraxin, the Syrian hamster female protein, determined by sequence analysis of a clone isolated from a hamster liver cDNA library (32); and (c) the previously reported protein sequence was based on alignment of peptides (8) by analogy with human CRP (33). No overlapping peptides were reported in the region in which the sequence derived from the cDNA and the protein sequence differ. Several single residue differences are noted elsewhere when the sequences determined from SAP-specific cDNA and the protein are compared. These could be due either to technical considerations in amino acid sequencing or represent microheterogeneity or allotypic variants. Overall, the amino acid sequence of human SAP derived from the nucleotide sequence analysis of the cDNA shows 54% homology with human CRP and 71% homology with Syrian hamster female protein.

SAP synthesis is directed by a messenger RNA species approximately one-half the size of the mRNA for CRP (7), although the monomeric subunit of each is similar in size. Hence the 3' and/or 5' untranslated regions for SAP are smaller than corresponding regions in the CRP mRNA. Native SAP is glycosylated (2, 4) so that, although it is likely that the signal peptide of preSAP is cleaved during postsynthetic processing, the mobility of the mature intracellular and secreted SAP monomers is slower than that of the primary translation product.

Availability of the SAP cDNA clones and a panel of human-rodent hybrid cell lines permitted localization of the SAP gene to chromosome 1. Moreover, both SAP and CRP cDNA probes hybridized to restriction endonuclease fragments of identical size in DNA from two individuals. Several possible explanations for this finding were considered: (a) the similarity in size of the fragments detected with the two probes was coincidental; (b) the DNA within the boundaries defined by both enzymes which contain coding regions for CRP and SAP are sufficiently conserved, so that identical fragments are generated even though the genes are not closely linked; and (c) both probes recognize a common fragment suggesting close

linkage of the SAP and CRP genes. The third possibility is most likely but cannot be established with certainty until genomic clones have been isolated and characterized.

Analysis of genomic structure of human SAP and CRP and a comparison with the structures of the corresponding genes in other species should provide insight into the molecular basis for the differences in regulation of SAP and CRP gene expression during the acute phase response. Comparison with the structure of the Syrian hamster female protein gene will be of particular interest because expression of this homologous gene is regulated by sex steroids as well as mediators of the acute phase response.

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