(ii) subsequent analysis of the developmental and tissue specific expression of an exogenous sequence in embryos containing the gene integrated at the same site.

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21. Supported by NSF grant PCM 80230077, an Institutional American Cancer Society grant, and NIH grant GM31479-01 (L.D.E.). We thank L. Etkin for technical assistance, Drs. S. Riggsby, M. A. Handel, K. Sirotkin, and F. Wilt for critical review of the manuscript, and F. Shrode for typing the manuscript.

21 January 1983; revised 30 March 1983

Isolation of Human C-Reactive Protein Complementary DNA and Localization of the Gene to Chromosome 1

Abstract. *With a synthetic oligonucleotide mixture as probe, complementary DNA clones of C-reactive protein were isolated from an adult human liver complementary DNA library. The clones ranged in size from 700 to 1100 base pairs and were identified by partial DNA sequence analysis. One complementary DNA clone was used as a probe for hybridization with human-rodent DNA's isolated from somatic cell hybrids and bound to nitrocellulose filters (Southern blot analysis) to assign the human C-reactive protein gene to chromosome 1.*

Originally C-reactive protein (CRP) was defined as a substance—observed in the plasma of patients with acute infections—that reacted with the C polysaccharide of *Streptococcus pneumoniae* (*1*). Human CRP is outstanding among the plasma proteins that increase in concentration after tissue injury or inflammation (the acute phase proteins). During the acute phase response, the serum concentration of CRP may increase 1000 times or more, exceeding by several orders of magnitude increases in the concentrations of the other acute phase proteins (*2*). Human CRP has been purified to homogeneity, its complete amino acid sequence has been determined (*3*), and considerable information on the biological effects of CRP in man and experimental animals has been obtained (*4*). The isolation of complementary DNA (cDNA) probes for the CRP sequence would permit analysis of the acute phase response at the molecular level and a detailed description of the structure, chromosomal location, and expression of the CRP gene.

A synthetic oligonucleotide mixture, comprising all 17-nucleotide DNA sequences that could code for the amino acid sequence of CRP between residues 141 and 146 (*3*), was generated by a solid-phase phosphotriester method, using a library of dimer anions (*5*). Residues 141 to 146 of the amino acid sequence of human CRP display minimal ambiguity in codon assignment and required synthesis of only eight oligonucleotide sequences (Fig. 1). The oligonucleotide mixture was labeled at the 5′ end with adenosine [γ-^{32}P]triphosphate, treated with T4 polynucleotide kinase (*6*), and used as a hybridization probe to screen approximately 25,000 human liver cDNA clones (*5*). Fifteen clones hybridized specifically to the oligonucleotide mixture. After colony purification, plasmid DNA was isolated from five of the positive clones by the cleared lysate method (*7*). The cDNA inserts derived from the purified plasmids by digestion with the restriction endonuclease Pst I ranged in size from 700 to 1100 base pairs. The 5′ and 3′ ends of the largest clone, pCRP1, were sequenced (*6*). The nucleotide and derived amino acid sequences of the 5′ end of this clone are shown (Fig. 1). These sequences at the 5′ end define residues 107 to 138 in human CRP (*3*), thus confirming pCRP1 as a CRP-specific cDNA clone. Restriction enzyme mapping and the 5′ DNA sequence established the relation between the clone pCRP1, the native protein, its messenger RNA (mRNA), and the synthetic oligonucleotide mixture (Fig. 1). The amino acid sequence of CRP comprises 187 residues (*3*), requiring an mRNA of at least 561 nucleotides, not including the 3′ and 5′ untranslated regions. Partial characterization of pCRP1 (Fig. 1) shows that this clone contains 243 nucleotides of the CRP coding sequence corresponding to the region from residue 107 to the carboxyl terminus and 600 nucleotides of the 3′ untranslated region.

The chromosomal location of a number of human genes has been established with the use of specific DNA clones as hybridization probes to identify human gene sequences in the Southern blot analysis of DNA samples isolated from

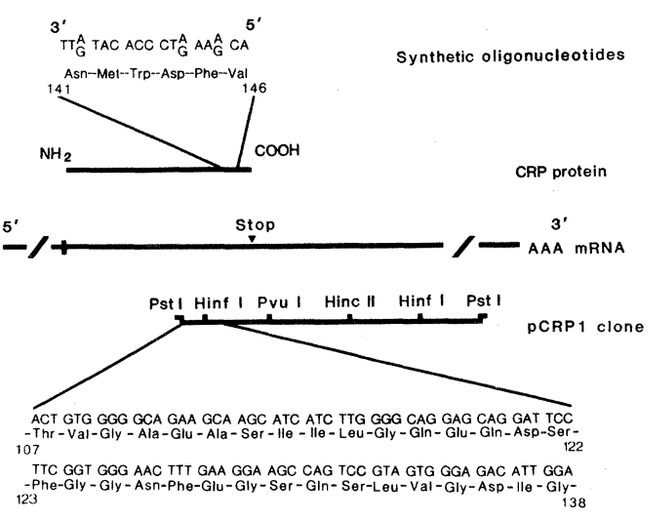

Fig. 1. Relation between the pCRP1 clone, the native C-reactive protein, its mRNA, and the synthetic oligonucleotide mixture. The 5′ nucleotide sequence and derived amino acid sequence establish the identity and position of the clone relative to the protein. Internal restriction endonuclease sites are Pvu I, Hinf I, and Hinc II.

somatic cell hybrids and bound to nitrocellulose filters (*8*). By this method, the CRP specific clone, pCRP1, was used to determine the chromosomal location of the CRP gene.

DNA was isolated from peripheral blood lymphocytes of ten unrelated human adults (*9*). Digestion of these DNA samples with the restriction endonuclease Bam HI and subsequent Southern blot analysis (*10*) with ^{32}P-labeled pCRP1 (*11*) as probe revealed a single band of 5.9 kilobases (kb) in all cases (data not shown). In similar experiments, DNA was isolated from mouse-human and hamster-human somatic cell hybrids containing a full complement of rodent chromosomes and a limited number of human chromosomes in varied combinations as previously determined by isoenzyme or chromosome analysis or both (*12*). DNA from each of the hybrids and rodent parent cells was digested with Bam HI and hybridized by the Southern blotting technique with ^{32}P-labeled pCRP1. The 5.9-kb band was present in 17 of the 24 somatic cell hybrid DNA samples analyzed. No hybridization was detected in DNA from the rodent cell lines (Fig. 2). The presence or absence of the 5.9-kb band in the Bam HI–digested DNA of the somatic cell hybrids is concordant only with the presence or absence of human chromosome 1 (Table 1). This therefore permits the assignment of the gene for CRP to chromosome 1.

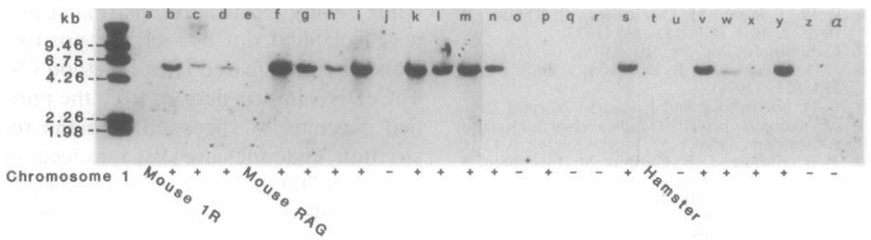

Fig. 2. Hybridization of pCRP1 to nitrocellulose filters bearing Bam HI–digested DNA from mouse-human and hamster-human somatic cell hybrids and their rodent parent cells. High molecular weight DNA was isolated (*9*), digested with the restriction endonuclease Bam HI, subjected to agarose gel electrophoresis, and transferred to nitrocellulose filters (*10*). The probe was ^{32}P-labeled by nick translation (*11*); hybridization to 10 μg of cleaved DNA bound to filters was performed (*15*). After hybridization, filters were washed in a solution of 30 m*M* NaCl and 3 m*M* sodium citrate at 65°C. The DNA samples represented in each track are (a) mouse parental cell line 1R; (b to d) mouse-human somatic cell hybrids SIR7-A2, -D1, and -G1; (e) mouse parental cell line RAG; (f to m) mouse-human somatic cell hybrids MOG2-A2, -A5, -B1, -C2, -D2, -E2, -E5, and -G1; (n to p) mouse-human somatic cell hybrids G24-B7, -A9, and -A4C1; (q and r) mouse-human somatic cell hybrids G17-11 and G17-15; (s): mouse-human somatic cell hybrid RRP3-6; (t) hamster parental cell line E36; (u to α) hamster-human somatic cell hybrids G35-A2, -A4, -D2, -D5, -D4, -E3, and -F3 (*14*). The presence or absence of human chromosome 1 in each cell line is denoted below each track. Molecular weight markers are Hind III–digested lambda phage.

Table 1. Segregation of hybridization of the DNA probe pCRP1 to Bam HI–digested DNA from 24 somatic cell hybrids. Concordant: (++) probe hybridizes, chromosome present; (−−) probe does not hybridize, chromosome not present. Discordant: (+−) probe hybridizes, chromosome not present; (−+) probe does not hybridize, chromosome present. Data are summarized from hybridization of pCRP1 to DNA from somatic cell hybrids (Fig. 2) previously analyzed for human chromosome content by karyotype and isoenzyme analysis (*12*). Data are unavailable for chromosomes 17 and X in cell lines G17-11 and G17-15, for chromosome 13 in cell line G17-15, and for chromosome 18 in cell line RRP-6.

Human chromosome	Hybridization of probe					
	Concordant		Discordant		Total	
	++	−−	+−	−+	Concordant	Discordant
1	17	7	0	0	24	0
2	7	4	10	3	11	13
3	12	2	5	5	14	10
4	11	4	6	3	15	9
5	12	3	5	4	15	9
6	13	1	4	6	14	10
7	12	4	5	3	16	8
8	6	3	11	4	9	15
9	9	6	8	1	15	9
10	12	2	5	5	14	10
11	10	3	7	4	13	11
12	15	2	2	5	17	7
13	13	2	4	4	15	8
14	14	2	3	5	16	8
15	12	5	5	2	17	7
16	13	5	4	2	18	6
17	13	3	4	2	16	6
18	11	1	5	6	12	11
19	14	0	3	7	14	10
20	10	2	7	5	12	12
21	15	2	2	5	17	7
22	10	4	7	3	14	10
X	13	4	4	1	17	5

Positive hybridization signals in three cell lines (data not shown) that contain rearrangements of chromosome 1 suggest that the CRP gene may be located on the proximal short arm or proximal long arm of the chromosome. The assignment of CRP to chromosome 1 will permit linkage analysis of CRP with one or more of the polymorphic loci (for example, phosphoglucomutase 1, the Duffy blood group, or antithrombin III) on this chromosome.

Two factors facilitated the generation, isolation, and identification of the cDNA clones described above. First, the complete amino acid sequence of human CRP is known (*3*), and from this information a synthetic oligonucleotide mixture could be synthesized for use as a probe to identify specific cDNA clones. Synthetic oligonucleotide mixtures have been used for the isolation of cDNA clones corresponding to sequences of several mRNA species of low abundance. These include human β_2 microglobulin (*13*); factor B, a human class III gene product of the major histocompatibility complex (*5*); and HLA-DR alpha chain, a class II gene product of the major histocompatibility complex (*14*). Second, the donor of the liver from which the mRNA used to construct the cDNA library (*5*) was prepared had sustained a severe injury 48 hours before the time of mRNA extraction. As expected from the time course of the acute phase response, the mRNA directed synthesis of high levels of CRP; hence the library contained the corresponding cDNA sequences.

The CRP-specific cDNA clone described above was used to localize the human CRP gene to chromosome 1. In addition this probe will permit analysis of the CRP gene structure, examination of transcription and posttranscriptional

processing of CRP RNA and quantification of mature mRNA. These studies should facilitate a detailed description of the molecular mechanisms controlling CRP biosynthesis during the acute phase response.

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17 January 1983; revised 5 April 1983

Bioactive Cardiac Substances: Potent Vasorelaxant Activity in Mammalian Atria

Abstract. *Mammalian atrial extracts possess natriuretic and diuretic activity. In experiments reported here it was found that atrial, but not ventricular, extract also causes relaxation of isolated vascular and nonvascular smooth muscle preparations. The smooth muscle relaxant activity of atrial extract was heat-stable and concentration-dependent and could be destroyed with protease. Rabbit aortic and chick rectum strips were used for the detection of atrial biological activity. The atrial activity was separated by column chromatography into two peaks having apparent molecular weights of 20,000 to 30,000 and less than 10,000. The atrial substance that copurified with the smooth muscle relaxant activity in both peaks caused natriuresis when injected into conscious rats. It appears that atria possess at least two peptides that elicit smooth muscle relaxation and natriuresis, suggesting an endogenous system of fluid volume regulation.*

Muscle fibers of the atria but not of the ventricles possess granules typical of protein secretory cells (*1*). The content and function of these granules remain unresolved; however, atrial granularity is enhanced by water deprivation and sodium deficiency (*2*). Since the atria are a site of fluid volume receptors (*3*), they would be an ideal site for the synthesis and release of substances that participate in the regulation of fluid volume.

The possibility that the atrial granule content regulates extracellular fluid volume and ionic concentration is supported by the observation that rat atrial homogenates or purified atrial granules cause natriuresis and diuresis when injected into rats (*4*). Rat ventricular homogenates have no such effect on kidney function. Initial efforts to characterize the atrial substance that causes natriuresis and diuresis have shown that the material is heat-stable and sensitive to protease digestion (*4*). Partial purification of the activity was achieved by fractionation on a Sephadex G-75 column (*5*). The activity eluted in fractions of low molecular weight (3600 to 5000 daltons) and high molecular weight (36,000 to 44,000 daltons). Efforts at purification have been hampered by the necessity to assay each fraction in an intact rat. Such preparations exhibit considerable variability, are time-consuming, and cannot readily be used to test multiple samples.

To determine whether rat atrial extracts facilitate sodium and fluid excretion by inducing renal prostaglandin synthesis, we injected the material into perfused hydronephrotic rabbit kidneys in vitro. Although prostaglandin was not released, renal resistance in the perfused kidneys was reduced. The ventricular extract was inactive. This renal vasodilation was not expected, since others have reported that the atrial extract does not alter renal blood flow or glomerular filtration rate (*4, 5*). We therefore initiated studies to ascertain the presence of a vasodilator in the atrial extracts, reasoning that an atrial substance that could alter sodium content, fluid volume, and vascular tone would be an ideal regulator of extracellular volume. Furthermore, we hoped to develop an in vitro smooth muscle assay that would enable the rapid, quantitative assessment of atrial biological activity to facilitate efforts at purification.

After screening numerous vascular segments, we found that the rabbit aorta strip maintained in tone by a continuous infusion of norepinephrine was a most reliable and sensitive assay tissue. Addition of rat atrial homogenate caused a rapid, transient (2 to 5 minutes) contraction of rabbit aorta strips, immediately followed by a prolonged relaxation (10 to 30 minutes). The ventricular extract caused the rapid contractile response but no vasorelaxation. The contractile activity in the atrial and ventricular homogenates was destroyed by boiling the extracts for 10 minutes. Atrial vasorelaxant activity was not affected by boiling for 3 to 10 minutes, but was abolished by trypsin (Fig. 1A). The atrial vasorelaxant activity was dose-dependent, with as little as 10 μl of extract (~ 2 percent of the total extract from a single rat atrium) capable of eliciting a response (Fig. 1B). Boiled atrial, but not ventricular, extract relaxed isolated chick rectum strips maintained in tone by a continuous infusion of carbachol (Fig. 1C). The intestinal smooth muscle activity of the atrial extract was heat-stable, trypsin-sensitive, and dose-dependent (Fig. 1C). The response of the chick rectum to the atrial extract lasted just 2 to 3 minutes, compared to 10 to 30 minutes for the rabbit aorta. Both preparations exhibited no sign of refractoriness after repeated injections of extract and were stable for up to 6 hours. The chick rectum preparation, therefore, provides a rapid and simple bioassay that allows for the testing of a large number of samples.

Atrial granules occur in several mammalian species, including man (*1*). We found that boiled human atrial extract,