corresponding to $J_{\beta 2}$-1 and the expressed J segment in pTβ2 are identical, suggesting that there are no polymorphic differences in $J_{\beta 2}$-1 segments of the individual whose DNA was used to make the genomic library and the individual whose thymus was used to make the cDNA library.

The nucleotide sequence of the 5′ C-region segment derived from pTβ2 and that derived from pTβ3 differed by two nucleotides (Fig. 3) as did the corresponding portions of the germline $C_{\beta 1}$ and $C_{\beta 2}$ genes. The nucleotide difference in the coding portions of $C_{\beta 1}$ and $C_{\beta 2}$ resulted in a change from Asn-Lys to Lys-Asn. This observation was unexpected because both plasmids contain J segments derived from the cluster of J segments encoded between $C_{\beta 1}$ and $C_{\beta 2}$. Subsequent nucleotide sequence analysis and restriction enzyme mapping demonstrated that pTβ2 contains the $C_{\beta 1}$ gene segment, whereas pTβ3 contains the $C_{\beta 2}$ segment (*17*).

Comparison of the pTβ2 cDNA with the structure of the locus for the C region of the β chain revealed an unexpected finding; the $J_{\beta 2}$-1 segment is joined to the $C_{\beta 1}$ segment. One explanation for this observation might have been the presence of a segment resembling $J_{\beta 2}$-1 in the $J_{\beta 1}$ cluster; however, the $J_{\beta 2}$-1 probe does not hybridize to the 9 kb of DNA 5′ of $C_{\beta 1}$ (*17*). We demonstrated (Fig. 2) that sequences 5′ of the $C_{\beta 1}$ segment are significantly different from sequences 5′ of $C_{\beta 2}$. Thus, there is only a single sequence like $J_{\beta 2}$-1 in the C-region locus and this segment is encoded 3′ of $C_{\beta 1}$.

Presumably, pTβ2 is encoded by an active gene in which the $J_{\beta 2}$-1 segment is 5′ of the $C_{\beta 1}$ segment. Since this gene structure is not encoded in the germline DNA, we suggest that it is formed during the somatic development of T cells. Perhaps a somatic recombination event involving an unequal crossover occurred during maturation of the cell expressing the pTβ2 messenger RNA (mRNA) and this recombination event placed $J_{\beta 2}$-1 5′ of $C_{\beta 1}$ (Fig. 4). Consistent with this hypothesis is the evidence that T-cell extracts contain an enzymatic activity that is able to promote homologous recombination in vitro (*19*).

The isolation of a β-chain cDNA derived from the $C_{\beta 1}$ segment and the $J_{\beta 2}$ cluster suggests an unexpected mechanism by which any J segment can be associated with either C_β segment. Since the $C_{\beta 1}$- and $C_{\beta 2}$-region genes are highly homologous, differing by six amino acid residues, the biological significance of these new forms of mRNA remains to be determined. Whether β-chain mRNA's utilizing one C-region segment are functional in one type of T cell while the other C_β segment is utilized in another T-cell subset remains an unanswered question. We do not yet know if this hypothesized somatic recombination occurs by a special mechanism or if this type of recombination occurs between all homologous genes during somatic development. We also do not know the frequency at which these unusual mRNA's are made in the thymus. The β-chain cDNA's derived from murine thymus do not have this structure (*7*). Eleven human β-chain cDNA's have been examined (*20*) (Fig. 3) and only one of them has the unexpected structure found in pTβ2. Analysis of active T-cell genes that have been formed by these unusual recombination events should provide some answers to these questions.

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Syrian Hamster Female Protein: Analysis of Female Protein Primary Structure and Gene Expression

Abstract. *The concentration in plasma of the female protein (FP) of the golden Syrian hamster is regulated by sex steroids and by mediators of the acute-phase response to tissue injury or inflammation. A complementary DNA (cDNA) clone corresponding to FP was isolated from a hamster liver cDNA library and used to determine the nucleotide sequence and derived amino acid sequence of native FP. The primary sequence of FP is 69 percent identical to human serum amyloid P component and 50 percent identical to human C-reactive protein. Evidence showed that sex-limited and acute-phase control of the FP gene is pretranslational. The FP protein is thus a useful model for investigating dual regulation of expression of a single gene.*

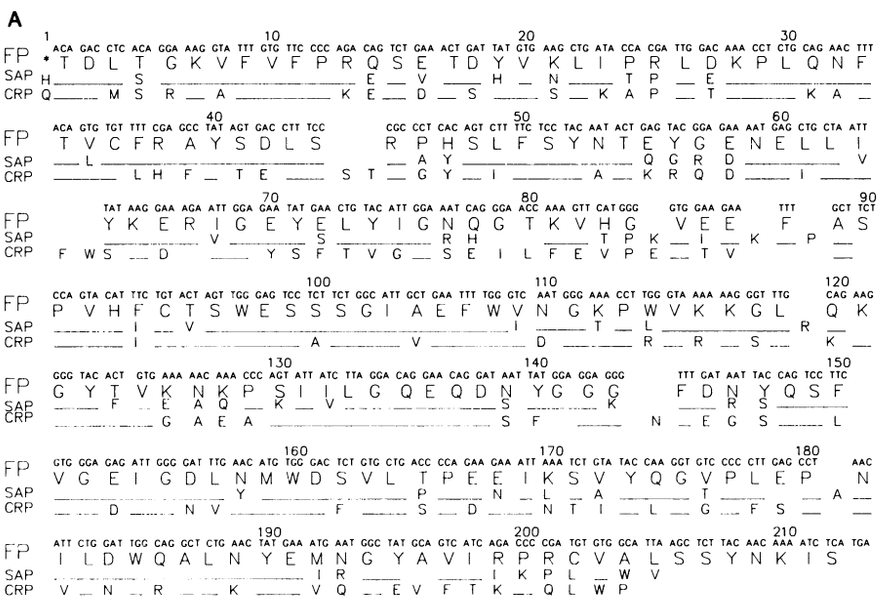
The plasma of golden Syrian hamsters contains a protein, designated female protein (FP), that is a member of the pentraxin family (*1*). Pentraxins, such as human C-reactive protein (CRP), serum amyloid P component (SAP), and other related proteins, have a characteristic subunit structure and are present in many species, including primitive invertebrates (*2*). Many pentraxins (for example, human CRP and murine SAP) are acute-phase proteins, which means that their concentrations in plasma increase after tissue injury or acute inflammation. The plasma concentration of FP in the Syrian hamster is under dual control; that is, concentrations are altered by sex steroids and by stimuli that elicit an acute-phase response. The concentration in plasma of female Syrian hamsters (0.5 to 3.0 mg/ml) is 100 to 1000 times greater than that in the plasma of normal males (4 to 20 μg/ml). Concentrations of FP increase by a factor of 5 to 10 in plasma of male hamsters during an inflammatory response but decrease by 50 percent in female hamsters after a similar acute-phase stimulus. Resting FP concentrations and the response to acute-phase stimuli are reversed by castration or by administration of sex steroids (*3*).

Complementary DNA (cDNA) clones corresponding to human CRP and SAP have been isolated (*4, 5*). Comparison of the structure and regulation of expression of the FP gene with those of other pentraxin genes would provide an opportunity to study the molecular basis of hormonal and acute-phase regulation of

plasma protein biosynthesis. Accordingly, we isolated an FP cDNA clone, determined its nucleotide sequence, and used it to probe the mechanism for dual regulation of FP gene expression.

Complementary DNA was generated from adult female hamster hepatic polyadenylated RNA and was cloned into pBR322 by means of methods described (*6*). The cDNA library was screened by filter colony hybridization (*7*) with an isotopically labeled human SAP–specific cDNA probe (*5*). Of the 30,000 tetracycline-resistant transformants screened, a plasmid containing a 720-base-pair (bp) cDNA insert (pFP1) was selected from 32 positive clones for restriction mapping and DNA sequence analysis (Fig. 1).

The selected clone contained the coding sequence for the entire mature FP and 60 bp of the 3′ untranslated region, but it did not include a poly(A) tail (Fig. 1). The FP amino acid sequence, derived from the pFP1 nucleotide sequence, shared appreciable homology with the derived amino acid sequence for human SAP (*5*) and with the amino acid sequence for human CRP (*8*). Discrepancies in amino acid homology between residues 68 and 116 of FP were noted when the FP sequence was compared with the amino acid sequence for human SAP (*9*). Peptides from the discrepant region of SAP had been aligned on the basis of their homology with CRP (*8*). Overall the sequence of FP is 69 percent identical to that of SAP and is 50 percent identical to that of CRP. More than half the amino acid mismatches can be explained by single nucleotide substitutions. A highly conserved region of se-



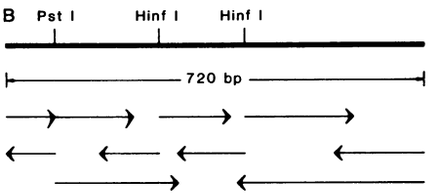
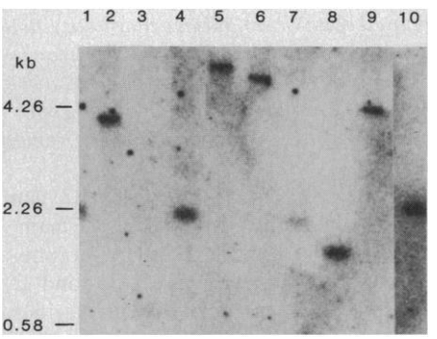


Fig. 1. Sequence analysis of pFP1. (A) Nucleotide sequence of pFP1 and the derived amino acid sequence, compared with the amino acid sequence for human CRP (*8*) and the derived amino acid sequence for human SAP (*5*). Regions of homology are indicated by lines connecting nonhomologous amino acids. The numbering system refers to FP amino acid residues, and gaps have been inserted in the depiction of FP, CRP, and SAP to maximize homology. (*) Amino acid position 1 has been shown to be perchloric acid (*1*). The small capital letters are the one-letter abbreviations for nucleotides: A, adenine; C, cytosine; G, guanine; T, thymine. The large capital letters are the one-letter abbreviations for amino acids. (B) The 720-bp excisable insert was mapped and found to contain an internal Pst I site and two Hinf I sites. Pst I digests of the plasmid containing the insert were subjected to 3′ labeling with terminal transferase and cordecypin deoxyadenosine [^{32}P]triphosphate. Hinf I digests were labeled at the 5′ ends with adenosine [γ-^{32}P]triphosphate and T_4 polynucleotide kinase (*15*). The DNA sequence of end-labeled fragments was determined by the method of Maxam and Gilbert (*15*). The sequence of regions overlapping Hinf I sites was confirmed by subcloning the larger Pst I fragment into an M13 vector (mp11) with subsequent dideoxynucleotide chain termination sequencing (*16*).



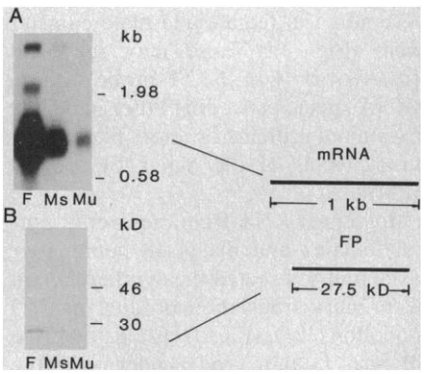


Fig. 2 (left). Analysis of restriction fragments of FP DNA. Agarose gel electrophoresis and transfer to nitrocellulose paper was performed on restriction digests of female hamster genomic DNA with the following enzymes: (lane 1) Sau 96, (lane 2) Bgl II, (lane 3) Hinf I, (lane 4) Stu I, (lane 5) Apa I, (lane 6) Pvu II, (lane 7) Hind III, (lane 8) Bst NI, (lane 9) Bam HI, and (lane 10) Xba I. These blots were then probed with nick-translated pFP1. Fig. 3 (right). (A) RNA blot analysis of FP-specific transcripts. Agarose-formaldehyde gel electrophoresis was performed after applying 5 μg of polyadenylated hepatic mRNA from each of the following hamsters: an unstimulated adult female (F), an adult male 48 hours after an intramuscular injection of turpentine (0.5 cm^3) (Ms), and an unstimulated adult male (Mu) (*17*). Quantities of RNA in each lane were similar when compared under ultraviolet light with ethidium bromide staining. The RNA blots were hybridized with gel-purified pFP1 treated with deoxycytosine [^{32}P]triphosphate (*11*). After hybridization, the blot was washed in 0.15*M* sodium chloride, 0.015*M* sodium citrate, and 0.1 percent sodium dodecyl sulfate at 54°C and then autoradiographed with Kodak XAR-5 film. (B) Immunoprecipitation of cell-free translation products. Total cytoplasmic RNA from hamsters was translated in a rabbit reticulocyte lysate containing [^{35}S]methionine (~1000 Ci/mmol) after optimizing the concentrations of RNA for each preparation to the conditions of the translation system (*14*). RNA was obtained from the following hamsters: an adult unstimulated female (F), an adult male 48 hours after an intramuscular injection of turpentine (0.5 cm^3) (Ms), and an unstimulated adult male (Mu). The volumes of translates were adjusted (21 ± 5 percent) to normalize for trichloroacetic acid–precipitable label and then were immunoprecipitated with an FP-specific antiserum and protein A (IgG Sorb; Enzyme Center). The size of isotopically labeled FP was estimated by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis according to Laemmli (*18*). Similar results were obtained with the antiserum described above (*13*).

quence homology occurs between amino acid residues 89 and 120 of FP when compared with CRP and SAP. Both amino- and carboxyl-terminus regions of FP have greater similarity to SAP than to CRP.

To examine whether the dual regulation of FP synthesis results from an effect of sex steroids and mediators of acute inflammation on a single gene or from their independent actions on two or more genes or gene copies, we analyzed restriction fragments of high molecular weight DNA by Southern blotting using FP-specific cDNA (*10*). Female hamster genomic DNA digests with ten restriction enzymes all resulted in single bands when Southern blots were probed with nick-translated pFP1 (Fig. 2) (*11*). In seven digests, restriction fragments containing FP-specific DNA were less than 4.26 kilobases (kb) in length. These data suggest that FP is encoded by a single gene locus.

The FP-specific cDNA insert was then used to examine basal concentrations of FP hepatic messenger RNA (mRNA) in normal female and male hamsters as well as in males 48 hours after induction of a sterile abscess with an intramuscular injection of turpentine (Fig. 3). FP-specific mRNA is 1 kb in length and is present in large quantities in female hamster liver in the resting state. FP mRNA was detected in small quantities in livers of unstimulated male hamsters and in increased quantities in the liver of stimulated male hamsters. Relative contents of FP mRNA corresponded to the relative concentrations of FP in serum of the animals from which liver mRNA was isolated (*12, 13*). In addition to the major species of FP-specific mRNA, at least two discrete species of higher molecular weight were noted in female and male hamster livers (Fig. 3). These may represent cross-reactivity of the FP probe with an mRNA species encoding other inducible sex-limited proteins or, more likely, precursor forms of the major FP-specific mRNA.

Messenger RNA from the liver of normal females and males 48 hours after tissue injury directed the synthesis of an FP primary translation product of 27.5 kilodaltons (kD) (Fig. 3) (*14*). Little or no FP was synthesized under cell-free translation conditions by mRNA isolated from plasma of a resting male hamster.

These findings support the utility of FP as a model for examining the regulation of protein synthesis by mediators of acute inflammation and by sex steroids. The evidence suggests that pretranslational regulation of FP synthesis is a function of these two factors.

Female protein shares greater amino acid sequence homology with human SAP than with CRP, even though the property of Ca^{2+}-dependent binding to phosphorylcholine is characteristic of both CRP and FP whereas the Ca^{2+}-dependent binding to agarose is shared by FP and SAP (*1*). Isolation of genomic clones for FP should help elucidate the structure of the FP gene and provide insight into control elements that take part in the regulation of FP biosynthesis by sex steroids and mediators of acute inflammation.

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A New Class of Endogenous Human Retroviral Genomes

Abstract. *Human DNA contains multiple copies of a novel class of endogenous retroviral genomes. Analysis of a human recombinant DNA clone (HLM-2) containing one such proviral genome revealed that it is a mosaic of retroviral-related sequences with the organization and length of known endogenous retroviral genomes. The HLM-2 long terminal repeat hybridized with the long terminal repeat of the squirrel monkey virus, a type D retrovirus. The HLM-2* gag *and* pol *genes share extensive nucleotide sequence homology with those of the M432 retrovirus (a type A–related retrovirus), mouse mammary tumor virus (a type B retrovirus), and the avian Rous sarcoma virus (a type C retrovirus). Nucleotide sequence analysis revealed regions in the HLM-2* pol *gene that were as much as 70 percent identical to the mouse mammary tumor virus* pol *gene. A portion of the putative HLM-2* env *gene hybridized with the corresponding region of the M432 viral genome.*

Some members of Retroviridae (*1*) can induce tumors in many species of mammals. Retroviruses classified by morphologic criteria as type A, B, C, and D viruses (*2*) are associated with various types of malignancy. Although many of these retroviruses induce tumors as infectious agents, they can also be transmitted as endogenous proviral genomes through the germline. Recent studies have revealed endogenous retroviral sequences in multiple copies within the genomes of animals and humans. One class of human endogenous retroviral sequences has been shown to be related to known mammalian type C viruses (*3*). A distinct class of human endogenous retroviral sequences has been detected and molecularly cloned with the mouse mammary tumor viral (MMTV) genome (prototype B virus) as a probe (*4*).

Evidence has accumulated showing that there have been genetic interactions between different retroviral classes during their evolution. Such interactions have been implied by immunologic cross-reactivities detected among respective structural proteins of these agents as well as by molecular hybridization (*5*). We now describe a human recombinant clone (HLM-2) that con-