Human inhibitor of the first component of complement, C1: Characterization of cDNA clones and localization of the gene to chromosome 11

(protease inhibitors/serpins)

ALVIN E. DAVIS III*†, ALEXANDER S. WHITEHEAD‡, RICHARD A. HARRISON§, ANDRE DAUPHINAIS*, GAIL A. P. BRUNS¶, MARCO CICARDI*, AND FRED S. ROSEN*

Division of *Immunology, †Nephrology, ‡Cell Biology, and ¶Genetics, Childrens' Hospital, 300 Longwood Avenue, Boston, MA 02115; and §Mechanisms in Tumour Immunity Unit, Medical Research Council Centre, Hills Road, Cambridge, United Kingdom

ABSTRACT   C1 inhibitor is a heavily glycosylated plasma protein that regulates the activity of the first component of complement (C1) by inactivation of the serine protease subcomponents, C1r and C1s. C1 inhibitor cDNA clones have been isolated, and one of these (pC1INH1, 950 base pairs) has been partially sequenced. Sequence analysis demonstrates that the C1 inhibitor is a member of the serpin "superfamily" of protease inhibitors. In the region sequenced, C1 inhibitor has 22% identity with antithrombin III, 26% with α₁-antitrypsin and α₁-antichymotrypsin, and 18% with human angiotensinogen. C1 inhibitor has a larger amino-terminal extension than do the other plasma protease inhibitors. In addition, inspection of residues that are invariant among the other protease inhibitors shows that C1 inhibitor differs at 14 of 41 of these positions. Thus, it appears that C1 inhibitor diverged from the group relatively early in evolution, although probably after the divergence of angiotensinogen. Southern blot analysis of BamHI-digested DNA from normal individuals and from rodent–human somatic cell hybrid cell lines (that contain a limited but varied human chromosome complement) was used to localize the human C1 inhibitor gene to chromosome 11.

C1 inhibitor regulates the activity of the first component of complement (C1) by inhibition of the proteolytic activity of its subcomponents C1r and C1s. This prevents the activation of C4 and C2 by C1s, thereby providing an important control over classical pathway activation. In addition to C1r and C1s, C1 inhibitor also inhibits several other serine proteinases including plasmin, kallikrein, and coagulation factors XIa and XIIa (1–4). Although each of these other enzymes is inhibited by other protease inhibitors, C1r and C1s are inhibited only by C1 inhibitor, which functions in a manner similar to the other plasma protease inhibitors in that it forms an apparent covalent complex with C1r and C1s in an equimolar ratio (5–7). This results in the dissociation of macromolecular C1, with the release of C1r–C1s–(C1 inhibitor)₂ complexes (8–10).

C1 inhibitor is the most heavily glycosylated plasma protein, containing about 35% carbohydrate by weight (11, 12). Its carbohydrate composition suggests that it has a number of O-glycosidic-linked oligosaccharide units (12). It consists of a single polypeptide chain with an apparent M_r of 105,000 as determined by NaDodSO₄/PAGE (5, 11–14). The amino-terminal amino acid sequence (40 residues) has been reported, as has the sequence (12 residues) surrounding the reactive center (12, 15). Although the mechanism of protease inhibition by C1 inhibitor is analogous to that of other plasma

protease inhibitors, the available sequence data do not yet confirm a relationship.

Hereditary angioneurotic edema is a dominantly inherited disease caused by apparent heterozygous deficiency of C1 inhibitor (16–18). There are two forms of the disease, types I and II (19). Type I is characterized by diminished plasma levels of an apparently normal C1 inhibitor protein. In type II hereditary angioneurotic edema, a dysfunctional C1 inhibitor protein that is unable to inhibit C1 activity is present at levels varying from 30% to 400% of normal, as measured antigenically.

To determine the structural basis of C1 inhibitor function, to define its evolutionary relationship to other protease inhibitors, and to analyze the molecular genetic basis of hereditary angioneurotic edema, we have begun to examine the C1 inhibitor gene. In this report, we have characterized a C1 inhibitor cDNA clone that represents approximately half the coding sequence of the protein, and we have compared the sequence of this clone with the analogous regions of other protease inhibitors. In addition, we provide evidence that the human C1 inhibitor gene is on chromosome 11.

MATERIALS AND METHODS

Protein and Peptide Isolation. C1 inhibitor was isolated from normal human plasma as described (12). Digestion with CNBr was done by incubation of salt-free reduced and alkylated protein with CNBr (100-fold molar excess over total methionine) in 70% formic acid. Peptides were separated by initial gel filtration on Sepharose CL-6B (2.5 × 170 cm) equilibrated in 0.2 M Tris/5 mM EDTA/6 M guanidine·HCl, pH 8.0. The pool of smaller peptides from this column was fractionated by HPLC on an Aquapore RP300 column (Brownlee Labs, Santa Clara, CA) equilibrated in 0.1% CF₃COOH and developed with a linear acetonitrile gradient to 70%.

Amino Acid Sequence Analysis. Automated Edman degradations were performed with a Beckman 890C sequencer modified with a cold trap. A 0.1 M Quadrol program was used (20). Conversion was performed with methanolic HCl (1 part acetyl chloride and 7 parts methanol at 65°C for 10 min). Phenylthiohydantoin derivatives were identified by HPLC using a Zorbax ODS column (DuPont Instruments) equilibrated in 0.01 M sodium acetate, pH 5.5/20% acetonitrile and developed with an acetonitrile gradient (21). Repetitive yields in all sequencing runs ranged from 92% to 95%.

Synthetic Oligonucleotides. Six C1 inhibitor CNBr peptides were subjected to sequence analysis (Table 1). One of these (CB5) was the amino-terminal peptide (12), while another

---

Abbreviations: C1, first component of complement; bp, base pair(s); kb, kilobase(s).

Table 1. Amino-terminal sequences of C1 inhibitor CNBr peptides

CB1:	Leu-Phe-Val-Glu-Pro-Ile-Leu-Glu-Val-Xaa-Leu
CB2:	Ala-Phe-Ser-Pro-Phe-Ser-Ile-Ala-Ser-Leu-Leu-Xaa-Gln-Val-Leu-Leu-Gly-Ala-Gly-Asn-Ala-Glu-Ala-Xaa-Asn-Xaa-Glu
CB3:	Asn-Ser-Lys-Lys-Tyr-Pro-Val-Ala-His-Phe-Ile-Asp-Gln-Thr-Leu-Lys-Ala-Lys-Val-Gly-Gln-Leu-Gln-Leu-Ser-His-Xaa-Leu-Ser-Leu-Val-Ile-Leu-Val-Pro-Gln-Asn-Leu-Xaa-Xaa-Xaa-Leu-Glu
CB4:	Gln-His-Gln-Thr-Val-Leu-Glu-Leu-Thr-Glu-Thr-Gly-Val-Glu-Ala
CB5:	Asn-Pro-Xaa-Ala-Thr-Ser-Ser-Ser-Ser-Gln-Asp
CB6:	Glu-Lys-Leu-Glu-Met*-Ser-Lys-Phe-Gln-Pro-Thr-Leu-Leu-Thr-Leu-Pro-Xaa-Ile-Lys-Val-Thr-Thr-Ser-Gln-Asp

*Cleavage apparently did not occur at this methionine. It was detected at the appropriate yield during sequencing, and no peptide with a sequence beginning at residue 6 was found.

(CB1, apparent M_r 45,000 on NaDodSO$_4$/PAGE) had an amino-terminal sequence beginning at residue 32 of the intact protein (12). From the amino-terminal sequences of two of the other four peptides, two hexapeptide sequences (His-Phe-Ile-Asp-Gln-Thr from CB3 and Met-Gln-His-Gln-Thr-Val from CB4) were chosen to construct oligonucleotide

mixtures for screening of the cDNA library. Each of these two mixtures contained 64 different 17-nucleotide-long oligonucleotides, comprising all sequences that could code for the respective hexapeptides. Oligonucleotides for screening and for use as nucleotide sequencing primers were synthesized either on a Biosearch Sam One automated

FIG. 1.   Nucleotide sequence of the C1 inhibitor-specific cDNA, the derived amino acid sequence, and comparison of the amino acid sequence with those of other human plasma proteinase inhibitors. The numbering used refers to amino acid numbering and is from Carrell and Boswell (35). C1 INH, C1 inhibitor; α_1 AT, α_1-antitrypsin; α_1 AC, α_1-antichymotrypsin; AT III, antithrombin III. The standard single-letter amino acid code is used. The C1 inhibitor hexapeptide sequences used for construction of synthetic oligonucleotides are enclosed, and the amino acid residues determined also by protein sequence analysis are underlined. The insertion in C1 inhibitor relative to the other proteinase inhibitors is bracketed. The reactive centers are enclosed with a dashed line.

nucleotide synthesizer or on an Applied Biosystems 380B DNA synthesizer.

Screening for and Isolation of C1 Inhibitor cDNA Clones. An adult human liver cDNA library containing more than 200,000 recombinant clones (23) was plated on 82-mm-diameter nitrocellulose filters at high density and screened as described (24). The oligonucleotide mixtures were 5'-end-labeled with $[\gamma^{-32}P]ATP$ and T4 polynucleotide kinase (Bethesda Research Laboratories). Hybridization was done at 40°C, and filters were washed in 0.9 M NaCl/90 mM sodium citrate/0.05% sodium pyrophosphate at 42, 46, and 50°C. After each wash, filters were dried and exposed overnight on Kodak XAR-5 film. Plasmids were isolated by the alkaline NaDodSO₄ method (25). The C1 inhibitor-specific cDNA inserts were excised with *Pst* I (New England Biolabs) and isolated by preparative agarose gel electrophoresis. The C1 inhibitor cDNA clone was sequenced by the dideoxy chain-termination method of Sanger (49) after subcloning in the phage vector M13 mp18.

Messenger RNA Isolation and RNA Blot Analysis. RNA was isolated from the human hepatoma cell line, Hep G2 (26) as described (27), was size-fractionated by agarose/formaldehyde gel electrophoresis (28), and was transferred to nitrocellulose filters. The 600-base-pair (bp) *Pst* I fragment of the C1 inhibitor cDNA clone (pC1INH1) was labeled by nick-translation (29) and used as a hybridization probe. Hybridization was in 10% dextran/50% formamide buffer at 42°C; final washing was at 65°C.

Chromosomal Localization. The somatic cell hybrids used have been described and characterized for human chromosome content by isozyme and cytogenetic techniques (30–32). They also have been analyzed with cloned DNA probes for 21 of the human autosomes and the X chromosome. DNA isolated (33) from human peripheral blood leukocytes, mouse RAG cells, hamster E36 cells, and somatic cell hybrids was digested with *Bam*HI and subjected to agarose gel electrophoresis, and Southern blots were done (34). These blots were hybridized with the 600-bp *Pst* I fragment of pC1INH1 radiolabeled by nick-translation (29). Filters were washed in 30 mM NaCl/3 mM sodium citrate at 65°C for 1 hr and exposed overnight with Kodak XAR-5 film.

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of C1 Inhibitor cDNA. Approximately 40,000 recombinant colonies were screened of which 4 were positive with both oligonucleotide mixtures; of these 1 (pC1INH1) was further characterized. This clone consisted of ≈932 bp and contained an internal *Pst* I site that divides the insert into a 3' 332-bp fragment and a 5' 600-bp fragment. The 332-bp fragment has been fully sequenced, and 325 nucleotides of the 600-bp fragment have been determined. The nucleotide and derived amino acid sequences are shown in Fig. 1. The amino acid sequence contained three of the six CNBr peptides. Amino acid sequences derived also from protein sequence analysis are underlined. The nucleotide and known amino acid sequences agreed at all positions. The clone contained the sequences that matched the two synthetic oligonucleotide sequences. These hexapeptides are enclosed in Fig. 1. The molecular weight of the C1 inhibitor polypeptide chain is ≈64,000, as determined from cell-free synthesis studies (unpublished data). From this molecular weight and the amino acid composition reported by Harrison (12), C1 inhibitor consists of about 580 amino acids. The cDNA insert thus represents slightly more than half the coding sequence for C1 inhibitor (932 of an estimated 1740 nucleotides).

The amino acid sequence derived from the nucleotide sequence is aligned in Fig. 1 with the homologous portions of three other known human plasma protease inhibitors that are

members of the serpin "superfamily" (22, 36–40). As shown, by comparison, the 3' end of the clone terminates 27–30 nucleotides 5' to the reactive centers of the other inhibitors (enclosed by a dashed line). The C1 inhibitor reactive center sequence is from Salvesen *et al.* (15). Although it has been assumed that C1 inhibitor was a member of this group, the previous limited sequence data did not show homology (12, 15). As shown in Fig. 1, C1 inhibitor is related to these other protease inhibitors and to the other members of the superfamily, such as ovalbumin and angiotensinogen. In the region shown, C1 inhibitor as aligned in Fig. 1 has 22% identity with antithrombin III and 26% with α_1-antitrypsin and α_1-antichymotrypsin. It also has 18% identity with human angiotensinogen (not shown) (41). This degree of homology is similar to but slightly less than the degree of homology (29% to 44%) among the other antiproteases over the same region. It is, however, somewhat greater than the degree of homology between angiotensinogen and each of the protease inhibitors. There are several interesting and perhaps significant differences observed in comparing C1 inhibitor with the other serpins.

The alignment shown in Fig. 1 is essentially that of Carrell and Boswell (35), with alignments based on the secondary structure of α_1-antitrypsin (42) and insertion of gaps penalized in regions of defined secondary structure. The first difference that is apparent on inspection of Fig. 1, combined with previous knowledge of the C1 inhibitor molecule, is that the C1 inhibitor has a larger amino-terminal extension than do most of the other serpins. The other serpins have 45–55 amino acid residues between the end of the derived C1 inhibitor sequence shown in Fig. 1 and the carboxyl termini and ≈130–180 residues between their amino termini and the beginning of the sequences shown in Fig. 1. In addition to the CNBr peptides included in Fig. 1 (CB3, CB4, and CB6 in Table 1), C1 inhibitor contains two larger CNBr peptides (CB1 and CB2 in Table 1) (43). The sequence of CB1 overlaps with the reported amino-terminal sequence, beginning at residue 32 from the amino terminus (12). This data, together with the homology and the known variation in amino-terminal extensions among the other serpins, indicate that these larger peptides are both amino-terminal to the sequence shown in Fig. 1. If it is assumed that C1 inhibitor has about 50 amino acid residues carboxyl-terminal to the sequence in Fig. 1, then there must be slightly more than 300 amino acids amino-terminal to the sequence shown. Thus, C1 inhibitor has an amino-terminal extension that is more than 100 residues longer than those of the other serpins. The relationship of this extension to the function of C1 inhibitor remains to be defined. Virtually all of the carbohydrate side chains are in this portion of the molecule (12, 43). There are no potential sites for N-linked oligosaccharide in the sequence in Fig. 1.
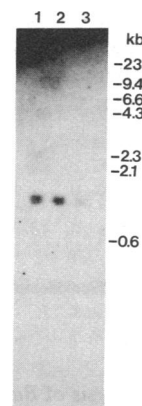

FIG. 2.   RNA blot analysis of C1 inhibitor-specific mRNA. Lanes: 1, 15 μg of RNA; 2, 10 μg of RNA; 3, 5 μg of RNA.

Table 2. Chromosomal localization of the gene for C1 inhibitor

Cell line	C1 Inh. band	Human chromosome																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
G17 11	−	−	−	−	−	+	+	+	−	−	+	−	+	+	+	−	−	+	+	+	+	+	−	−
G17 5	−	±	−	−	+	−	+	−	+	−	+	−	−	+	+	+	−	−	−	+	−	−	+	−
G24 A4	±	±	R	−	−	+	+	+	−	+	−	±	+	+	+	+	−	−	−	+	−	+	−	−
G35 D3	−	−	R	−	+	−	−	+	+	+	+	−	−	−	±	−	+	+	−	+	+	+	+	−
G35 A4	−	+	−	+	+	−	+	−	+	+	−	−	−	−	+	−	+	−	−	+	+	+	−	+
G35 D5	−	+	+	+	−	−	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	−	+	−
G46 C2	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	+
G35 C4	−	−	−	−	−	−	+	−	−	R	−	−	+	−	+	−	−	−	±	+	+	+	−	−
G89 E5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
G35 E3	+	−	−	−	−	+	+	−	+	−	−	+	+	+	+	−	+	−	+	+	+	+	+	−
G35 A5	+	R	R	+	+	−	+	+	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−
G35 D2	+	+	−	−	−	+	+	−	−	±	−	+	+	−	−	+	+	−	±	+	+	−	+	−
G35 A2	+	−	−	+	+	−	+	−	−	−	−	+	−	−	+	−	−	−	−	+	+	+	+	±
G35 B5	+	−	−	−	−	−	R	+	−	−	−	+	R	+	−	−	−	+	−	+	+	−	−	−
G24 B5	+	+	−	+	+	−	−	+	−	−	+	+	+	+	+	−	+	−	−	+	−	+	+	+
G35 C1	+	−	−	R	+	−	R	+	−	−	+	+	R	−	+	+	+	−	+	+	−	−	+	−
G35 F5	+	±	−	+	+	−	+	−	−	+	−	+	−	+	+	−	+	+	+	+	±	+	+	+
G35 D4	+	+	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+
Concordant		9	7	11	10	10	9	9	6	7	7	18	10	10	9	10	12	7	9	11	9	9	12	8
Discordant		6	8	5	7	7	6	8	11	8	10	0	5	7	7	7	5	10	6	6	7	8	5	8

The designations are: +, presence of the 6.0-kb C1 inhibitor BamHI fragment; −, absence of the fragment; ±, weakly positive signal for the above fragment on Southern blot analysis. Similar designations are used for the presence or absence of human chromosomes in the hybrid lines. The designation "R" indicates a chromosome rearrangement as evidenced by cytogenetic techniques or disruption of a known syntenic group. The chromosome 19 column represents the 19q⁺ translocation chromosome present in the hybrids, and the X chromosome column represents the intact X and the Xq- translocation chromosome (31, 32). A single hybrid (not shown) hybridized very weakly with the 6.0-kb C1 inhibitor BamHI fragment but failed to hybridize with two different chromosome 11-derived DNA probes. It is likely that this hybrid has retained only a fragment of chromosome 11 including the C1 inhibitor locus. "Concordant" indicates the sum of cell lines that contain the particular chromosome and are positive for the 6.0-kb C1 inhibitor-specific band or are negative for both. Discordant cell lines are those in which the chromosome in question is present and the 6.0-kb band is absent or in which the particular chromosome is absent and the 6.0-kb band is present. Inh., inhibitor.

Another difference between C1 inhibitor and the other serpins is apparent on examination of invariant residues observed when the sequences are aligned. There are 41 amino acid residues that are identical among the other three human plasma protease inhibitors. C1 inhibitor has a different amino acid at 14 of these positions. Similar analysis of each of the antiproteases in comparison with the other three shows appreciably less variation, with the exception of antithrombin III, which shows 11 differences among 37 positions that are identical in C1 inhibitor, α₁-antitrypsin, and α₁-antichymotrypsin. When this comparison is extended to the entire superfamily (the above inhibitors plus chicken ovalbumin, chicken gene Y, mouse contrapsin, mouse and baboon α₁-antitrypsin, and baboon and human angiotensinogen), C1 inhibitor differs at 3 of 11 positions that are invariant in the other serpins. The only other member that differs at any position that is invariant in the others is angiotensinogen. This is not surprising because it is obviously more distantly related to the other members of the group.

In addition to the above, C1 inhibitor shows two other variations. Between positions 350 and 360, it shows an insertion of four to six amino acid residues relative to the other serpins (bracket in Fig. 1). Also, proline-351, eight residues prior to this insertion, is within a region that in α₁-antitrypsin is in an α-helix. However, because of the insertion, the alignment through this segment (which shows a low degree of homology) may not be correct. This proline residue may in fact align with the prolines in antithrombin III and α₁-antichymotrypsin (position 348), which are just at the beginning of the α-helix (35).

RNA Blot Analysis. A C1 inhibitor mRNA of ≈1500 bp (compared with DNA markers) was identified in RNA isolated from a human hepatoma cell line (Hep G2) (Fig. 2) and from cultured human monocytes (not shown). A mRNA with a minimum size of 1740 bp would be expected from the size of the C1 inhibitor polypeptide. The C1 inhibitor mRNA, when compared with 18S and 28S RNA as markers, had a calculated length of 2100 bp. Thus, it is compatible with the expected size.
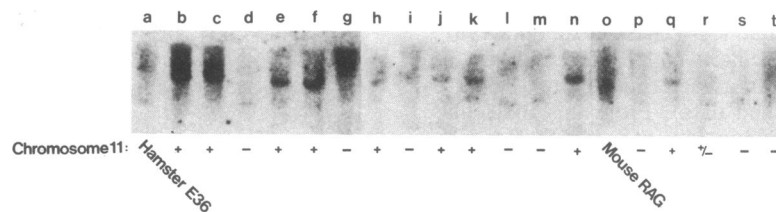
Chromosomal Localization of the Human C1 Inhibitor Gene.

FIG. 3. Southern blot analysis of BamHI-digested DNA from rodent–human somatic cell hybrids and rodent parent cell lines. The DNA samples in lanes: a, hamster parent cell line E36; b–l, hamster–human hybrids G35-B5, -C1, -C4, -A2, -D2, -D3, -D4, -D5, -E3, -F5, -A4; m, hamster–human hybrid G89-E5; n, hamster–human hybrid G35-A5; o, mouse parent cell line RAG; p–t, mouse–human hybrids G46-C2, G24-B5, G24-A4, G17-5, G17-11. The 6.0-kb band represents human C1 inhibitor. The presence or absence of chromosome 11 is shown below each track.

Somatic cell hybrids between human leukocytes and mouse RAG or hamster E36 cells were analyzed for the presence of the C1 inhibitor gene. These hybrids contain a limited but varied human chromosome complement, with a full rodent chromosome complement. Southern blot analysis of *Bam*HI-digested DNA isolated from peripheral blood leukocytes from seven normal individuals revealed a fragment of ≈6.0 kilobases (kb). This band was not detected in the DNA from the mouse RAG cells or the hamster E36 cells. DNA isolated from the somatic cell hybrids was also digested with *Bam*HI and subjected to Southern blot analysis. The presence or absence of the 6.0-kb C1 inhibitor-specific band correlated only with the presence or absence of chromosome 11 (Table 2, Fig. 3), thus allowing the assignment of the human C1 inhibitor gene to this chromosome. The gene for α_1-antitrypsin maps to chromosome 14 (44), and that for anti-thrombin III maps to chromosome 1 (45). Thus, although these proteinase inhibitors are ancestrally related, they do not constitute a linkage group on the same chromosome in man.

C1 inhibitor thus is a member of the serpin superfamily of serine protease inhibitors and related proteins. Based on comparison of the available sequences, C1 inhibitor very likely diverged from the group relatively early, although after the divergence of angiotensinogen. Interestingly, however, the structure of the genes for angiotensinogen and α_1-antitrypsin are remarkably similar in that each has four introns and the position of the introns is the same in the two genes (39, 46). The gene for ovalbumin, on the other hand, has seven introns that are arranged differently within the gene (47, 48). Preliminary Southern blot analysis of C1 inhibitor genomic clones indicate that the gene is at least 7–8 kb (unpublished data), but the gene structure is not yet known. Comparison of the structure of the gene for C1 inhibitor with the other genes may help to clarify the relationships among them.

Note Added in Proof. Since this manuscript was submitted, sequence analysis of other cDNA clones has shown that there are 42 additional amino acids between the end of the sequence shown in Fig. 1 and the carboxyl terminus of the protein. The sequence of the active center region agrees completely with that reported by Salvesen *et al.* (15), as shown in Fig. 1. In addition, these data indicate that the sequence at residues 427–431 (Glu-Thr-Gly-Val-Glu) is in the correct position and has a sequence compatible with that of the "hinge region" preceding the active center in serpins.

1. Ratnoff, O. D., Pensky, J., Ogston, D. & Naff, G. B. (1969) *J. Exp. Med.* **129**, 315–331.
2. Forbes, C. D., Pensky, J. & Ratnoff, O. D. (1971) *J. Lab. Clin. Med.* **76**, 809–815.
3. Gigli, I., Mason, J. W., Colman, R. W. & Austen, K. F. (1970) *J. Immunol.* **104**, 574–581.
4. Schreiber, A. D., Kaplan, A. P. & Austen, K. F. (1973) *J. Clin. Invest.* **52**, 1394–1401.
5. Harpel, P. C. & Cooper, N. R. (1975) *J. Clin. Invest.* **55**, 593–604.
6. Sim, R. B., Reboul, A., Arlaud, G. J., Villiers, C. L. & Colomb, M. G. (1979) *FEBS Lett.* **97**, 111–115.
7. Sim, R. B., Arlaud, G. J. & Colomb, M. G. (1980) *Biochim. Biophys. Acta* **612**, 433–449.
8. Sim, R. B., Arlaud, G. J. & Colomb, M. G. (1979) *Biochem. J.* **179**, 449–457.
9. Ziccardi, R. J. & Cooper, N. R. (1979) *J. Immunol.* **123**, 788–792.
10. Laurell, A.-B., Martensson, U. & Sjoholm, A. G. (1976) *Acta Pathol. Microbiol. Scand.* **84**, 455–464.
11. Haupt, H., Heimburger, N., Kranz, T. & Schwick, G. (1970) *Eur. J. Biochem.* **17**, 254–261.
12. Harrison, R. A. (1983) *Biochemistry* **22**, 5001–5007.
13. Pensky, J., Levy, L. R. & Lepow, I. H. (1961) *J. Biol. Chem.* **236**, 1674–1679.
14. Reboul, A., Arlaud, G. J., Sim, R. B. & Colomb, M. G. (1977) *FEBS Lett.* **79**, 45–50.
15. Salvesen, G. S., Catanese, J. J., Kress, L. F. & Travis, J. (1985) *J. Biol. Chem.* **260**, 2432–2436.
16. Osler, W. (1888) *Am. J. Med. Sci.* **95**, 362–367.
17. Donaldson, V. H. & Evans, R. R. (1963) *Am. J. Med.* **35**, 37–44.
18. Landermann, N. S., Webster, M. E., Becker, E. L. & Ratcliffe, H. E. (1962) *J. Allergy* **33**, 330–341.
19. Rosen, F. S., Charache, P., Pensky, J. & Donaldson, V. (1965) *Science* **148**, 957–958.
20. Brauer, A. W., Margolies, M. N. & Haber, E. (1975) *Biochemistry* **14**, 3029–3035.
21. Zalut, C., Henzel, W. S. & Harris, H. W. (1980) *J. Biochem. Biophys. Methods* **3**, 11–30.
22. Petersen, T. E., Dudek-Wojciechowska, G., Sottrup-Jensen, L. & Magnussen, S. (1979) in *The Physiological Inhibitors of Coagulation and Fibrinolysis*, eds. Collen, D., Wiman, B. & Verstraete, M. (Elsevier-North Holland Biomedical Press, Amsterdam), pp. 43–54.
23. Woods, D. E., Markham, A. F., Ricker, A. T., Goldberger, G. & Colten, H. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5661–5665.
24. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
25. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7**, 1513–1523.
26. Knowles, B. B., Howe, C. C. & Aden, D. P. (1980) *Science* **209**, 497–499.
27. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
28. Lehrach, H., Diamond, D., Wezney, J. M. & Boedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
29. Jeffreys, A. J. & Flavell, R. A. (1977) *Cell* **12**, 429–439.
30. Latt, S. A., Willard, H. F. & Gerald, P. S. (1976) *Chromosoma (Berlin)* **57**, 135–153.
31. Brook, J. D., Shaw, D. J., Meredith, L., Bruns, G. A. P. & Harper, P. S. (1984) *Hum. Genet.* **68**, 282–285.
32. Bruns, G. A. P., Mintz, B. J., Leary, A. C., Regina, V. M. & Gerald, P. S. (1979) *Biochem. Genet.* **17**, 1031–1059.
33. Gross-Bellard, M., Oudet, P. & Chambon, P. (1977) *Eur. J. Biochem.* **36**, 32–38.
34. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
35. Carrell, R. W. & Boswell, D. R. (1986) in *Proteinase Inhibitors*, eds. Barrett, A. & Salvesen, G. (Elsevier-North Holland Biomedical Press, Amsterdam), in press.
36. Carrell, R. W., Jeppsson, J.-O., Laurell, C.-B., Brennan, S. O., Owen, M. C., Vaughan, L. & Boswell, D. R. (1982) *Nature (London)* **298**, 329–334.
37. Hunt, L. T. & Dayhoff, M. O. (1980) *Biochem. Biophys. Res. Commun.* **95**, 864–871.
38. Doolittle, R. F. (1983) *Science* **222**, 417–419.
39. Tanaka, T., Ohkubo, H. & Nakanishi, N. (1984) *J. Biol. Chem.* **259**, 8063–8065.
40. Chandra, J., Stackhouse, R., Kidd, V. J., Robson, K. J. H. & Woo, S. L. C. (1983) *Biochemistry* **22**, 5055–5061.
41. Kageyama, R., Ohkubo, H. & Nakanishi, S. (1984) *Biochemistry* **23**, 3603–3609.
42. Loebermann, H., Tokuoka, R., Deisenhofer, J. & Huber, R. (1984) *J. Mol. Biol.* **177**, 531–556.
43. Harrison, R. A. & Rosen, F. S. (1982) *Mol. Immunol.* **19**, 1374 (abstr.).
44. Cox, D. W., Markovic, V. D. & Teshima, I. E. (1982) *Nature (London)* **297**, 428–430.
45. Kao, F. T., Morse, H. G., Law, M. L., Lidsky, A., Chandra, T. & Woo, S. L. C. (1984) *Hum. Genet.* **67**, 34–36.
46. Kidd, V. J., Wallace, R. B., Itakura, K. & Woo, S. L. C. (1983) *Nature (London)* **304**, 230–234.
47. Gannon, D. F., O'Hare, K., Perrin, F., LePennec, J. P., Benoist, C., Cochet, M., Breathnach, R., Royal, A., Garapin, A., Cami, B. & Chambon, P. (1979) *Nature (London)* **278**, 428–434.
48. O'Malley, B. W., Roop, D. R., Lai, E. C., Nordstrom, J. L., Catterall, J. F., Swanneck, G. E., Colbert, D. A., Tsai, M. J., Dugaiczyk, A. & Woo, S. L. C. (1979) *Recent Prog. Horm. Res.* **35**, 1–46.
49. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.