Fibrinogen
Structural Variants and Interactions
Volume 3

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PREFACE

This volume contains the contributions to two symposia on fibrinogen. The first symposium, which was entitled "Abnormal Fibrinogens as Probes of Structure-Function Relationships", was held at Stockholmsmässan (Stockholm International Fairs) in Älvsjö outside Stockholm on July 8th, 1983, as a part of the IX. International Congress on Thrombosis and Haemostasis. The second symposium, which was entitled "Fibrinogen-Structure and Function", was held at the Berzelius Laboratory of the Karolinska Institute in Stockholm on July 9th and 10th, 1983, as a Satellite Symposium of the IX. International Congress. Approximately 150 scientists from all parts of the world participated in the symposia.

Several European Fibrinogen Workshops had already been held during the previous 10 years, some of them in Martinsried outside München, the most recent one in Noordwijkerhout, The Netherlands in May 1982. In Stockholm many scientists from outside Europe participated for the first time.

The purpose of the present symposia was to review and summarize recent progress in fibrinogen research and to enable scientists interested in the various aspects of fibrinogen to meet and discuss. Obviously, the titles of the contributions reflect which research areas are regarded as being currently of special interest. Thus, many presentations dealt with structural-functional variants of fibrinogen and with the interaction between fibrinogen and fibrin, various proteolytic enzymes or various cell types. At the Satellite Symposium an introductory lecture was given by the President of the main congress, Professor Birger Blombäck.

The organizers of the symposia wish to express their sincere gratitude to the Executive Committee of the IX. International

Congress for support and advice and to several members of the Staff of the Blood Coagulation Research Department at the Karolinska Institute for invaluable help with the local organisation. Most generous financial support was given by the companies Pharmacia, Kabi, Serono and Imco. The excellent secreterial help of Mrs. T. Nieberle and the cooperation of Dr. R. Weber and the publisher, Walter de Gruyter, are gratefully acknowledged.

This fibrinogen conference was held in a building named after J. Berzelius, who was the first professor of chemistry at the Karolinska Medico-Surgical Institute and who 145 years ago to the day, i.e. July 10th, invented the name "protein" and used it for the first time for fibrin! The organizers hope that the conference participants will remember the exceptionally hot summer days for the many important scientific contributions made and perhaps also for the delightful banquet that was given in the Stockholm City Hall, with the more personal contributions by professors John Shainoff, Staffan Magnusson and Jack Hawiger, dealing with such, so far unpublished, topics as how to recognize enemies at meetings, slide projection in the dark and which molecules are ugly and which are beautiful.

München, Stockholm, Boston and Uppsala, October 1985

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Earlier publications in this series:

Fibrinogen
Recent Biochemical and Medical Aspects
Volume 1
Editors: A. Henschen, H. Graeff and F. Lottspeich
Walter de Gruyter, Berlin • New York, 1982

Fibrinogen
Structure, Functional Aspects and Metabolism
Volume 2
Editors: F. Haverkate, A. Henschen, W. Nieuwenhuizen and P.W. Straub
Walter de Gruyter, Berlin • New York, 1983

Forthcoming publication:

Fibrinogen
Fibrin Formation and Fibrinolysis
Volume 4
Editors: D.A. Lane, A. Henschen and M.K. Jasani
Walter de Gruyter, Berlin • New York, 1986

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I. A HISTORICAL NOTE

TWO THOUSAND YEARS OF FIBRINOGEN RESEARCH AND EVIDENCE FOR FIBRIN BEING THE FIRST PROTEIN

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Blood coagulation has attracted the interest of scientists, physicians, philosophers and even theologists for several thousand years. Already very long time ago blood and blood flow were considered as essential for life. A few examples of the central role of blood clotting and fibrin(ogen) in science and philosophy will be presented.

About 2300 years ago

Ever since Hippokrates and Aristoteles and until less than 150 years ago all life processes were believed to take place in the blood, and for a long time period all living and dead things were thought to be composed of the four elements: air, fire, earth and water. According to ancient medical theory (the humoral pathology) the body contains four fluids (humors) and the right proportion or mixing of them constitutes health, but an improper distribution constitutes disease. The four humors were all believed to be contained in the clotted blood and were also regarded as closely related to the four elements, the four temperaments, the four ages, a.s.o. (Fig. 1). The four components observed in the clotted blood were: the light red, oxygen-rich part of the clot - "blood", the dark red, oxygen-deficient part - "black bile", the yellowish

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Components of clotted blood	Red blood cells, oxidised form	Serum	Red blood cells, reduced form	Fibrin
Colours	Red	Yellow	Black	White
Humors	Blood	Yellow bile	Black bile	Phlegm
Elements	Air	Fire	Earth	Water
Temperaments	Sanguinic	Choleric	Melancholic	Phlegmatic
Ages	Child	Adolescent	Adult	Old
Seasons	Spring	Summer	Fall	Winter
Organs	Heart	Liver,Stomach	Milt	Brain,Bladder

Fig. 1. The four humors, elements, temperaments, a.s.o. and their relation to the components of clotted blood.

serum - "yellow bile" and the white layer on the surface of the clot - "phlegm". It is obvious that the "phlegm" corresponds to fibrin in modern nomenclature. Already at the time of Hippokrates it was known that many diseases were accompanied by increased amounts of "phlegm", and for thousand of years to follow the main medical diagnostic method consisted in the analysis of the patient's clotted blood, the most important therapeutic method being the attempts to remove the excess of "phlegm".

About 1300 years ago

In many religions blood is regarded as having special, philosophically significant properties. Thus, the Koran contains the following text, which is a part of Mohammed's first revelation: "Recite thou, in the name of thy Lord who created; created man from clots of blood".

About 150 years ago

Relatively pure preparations of the white, fibrous material from blood clots, now named fibrin, and of its precursor from unclotted blood, soon after named fibrinogen, were analysed for chemical composition and other properties by several scientists, among them Berzelius, Mulder and Virchow. It occurred to Berzelius that fibrin, albumin and "probably the coloured material", i.e. hemoglobin, contained the same kind of oxidised basic component, and as it seemed less convenient to name this component only after fibrin, he proposed the word protein, derived from primary or principal (Fig. 2). Thus, fibrin was the first substance designated as a protein!

Nowadays

Our understanding of blood coagulation and fibrin(ogen) is steadily increasing. At present we have reached the stage where the complete covalent structures of fibrinogen (Fig.s 3 and 4) and many other hemostasis-related proteins are known. For many of these proteins at least models for their spatial structures and for structure-function relationships have been conceived; their various interactions have been studied in great detail. Which kind and degree of comprehension will be achieved in the future?

Fig. 2. (next page) Excerpt from one of Berzelius' letters to G.J. Mulder in Rotterdam, the original letter being considerably longer. From: Jac. Berzelius Bref, II:2 (Söderbaum, H.G., ed.), Almquist & Wiksell, Uppsala, 1916.

Stockholm, le 10 juillet 1838.

Monsieur et ami,

Lorsqu'on jouit d'une correspondance aussi intéressante que la votre, et qu'on différe de répondre, il faut avoir des raisons valables. Voici les miennes. J'ai pendant les dernières 3 semaines de juin et la première de juillet pris les eaux de Spa, et pendant ce temps-là j'ai renoncé aux occupations scientifiques pour la promenade et le mouvement dans l'air libre, ce dont j'avais furieusement besoin....

Je reviens à présent à vos travaux sur la fibrine. Il est sans doute de la plus haute importance que la substance organique, séparée du soufre et du phosphore, se trouve être la même pour la fibrine et l'albumine. Mais en même temps il devient nécessaire d'étudier ses propriétés à l'état isolé et de déterminer son poids atomique.....je présume que l'oxyde organique, qui est la base de la fibrine et de l'albumine (et auquel il faut donner un nom particulier p.ex. protéine) est composé d'un radical ternaire, combiné avec de l'oxygène dans quelqu'un de ces rapports simples que la nature inorganique nous présente....

Le nom protéine que je vous propose pour l'oxyde organique de la fibrine et de l'albumine, je voulais le dériver de πρωτειος, parce qu'il parait être la substance primitive ou principale de la nutrition animale que les plantes préparent pour les herbivores, et que ceux-ci fournissent ensuite aux carnassiers. En dérivant le nom du mot grec pour la fibre, il serait moins convenable, puisque l'oxyde organique est la base aussi de l'albumine et probablement de la matière colorante, ainsi que d'autres encore.

Mais il est temps de finir mon griffonage. J'attents avec bien de l'intérêt d'avoir de vos nouvelles.

Votre dévoué

J. Berzelius

Human fibrinogen Aα-chain:

```
  1 A D S G E G D F L A E G G G V R+G P R V V E R H Q S A C K D S D W P F C S D E D
 41 W N Y K C P S G C R M K G L I D E V N Q D F T N R I N K L K N S L F E Y Q K N N
 81 K D S H S L T T N I M E I L R G D F S S A N N R D N T Y N R V S E D L R S R I E
121 V L K R K V I E K V Q H I Q L L Q K N V R A Q L V D M K R L E V D I D I K I R S
161 C R G S C S R A L A R E V D L K D Y E D Q Q K Q L E Q V I A K D L L P S R D R Q
201 H L P L I K M K P V P D L V P G N F K S Q L Q K V P P E W K A L T D M P Q M R M
241 E L E R P G G N E I T R G G S T S Y G T G S E T E S P R N P S S A G S W N S G S
281 S G P G S T G N R N P G S S G T G G T A T W K P G S S G P G S T G S W N S G S S
321 G T G S T G N Q N P G S P R P G S T G T W N P G S S E R G S A G H W T S E S S V
361 S G S T G Q W H S E S G S F R P D S P G S G N A R P N N P D W G T F E E V S G N
401 V S P G T R R E Y H T E K L V T S K G D K E L R T G K E K V T S G S T T T T R R
441 S C S K T V T K T V I G P D G H K E V T K E V V T S E D G S D C P E A M D L G T
481 L S G I G T L D G F R H R H P D E A A F F D T A S T G K T F P G F F S P M L G E
521 F V S E T E S R G S E S G I F T N T K E S S S H H P G I A E F P S R G K S S S Y
561 S K Q F T S S T S Y N R G D S T F E S K S Y K M A D E A G S E A D H E G T H S T
601 K R G H A K S R P V
```

Human fibrinogen Bβ-chain:

```
  1 Z G V N D N E E G F F S A R+G H R P L D K K R E E A P S L R P A P P P I S G G G
 41 Y R A R P A K A A A T Q K K V E R K A P D A G G C L H A D P D L G V L C P T G C
 81 Q L Q E A L L Q Q E R P I R N S V D E L N N N V E A V S Q T S S S S F Q Y M Y L
121 L K D L W Q K R Q K Q V K D N E N V V N E Y S S E L E K H Q L Y I D E T V N S N
161 I P T N L R V L R S I L E N L R S K I Q K L E S D V S A Q M E Y C R T P C T V S
201 C N I P V V S G K E C E E I I R K G G E T S E M Y L I Q P D S S V K P Y R V Y C
241 D M N T E N G G W T V I Q N R Q D G S V D F G R K W D P Y K Q G F G N V A T N T
281 D G K N Y C G L P G E Y W L G N D K I S Q L T R M G P T E L L I E M E D W K G D
321 K V K A H Y G G F T V Q N E A N K Y Q I S V N K Y R G T A G N A L M D G A S Q L
361 M G E N+R T M T I H N G M F F S T Y D R D N D G W L T S D P R K Q C S K E D G G
401 G W W Y N R C H A A N P N G R Y Y W G G Q Y T W D M A K H G T D D G V V W M N W
441 K G S W Y S M R K M S M K I R P F F P Q Q
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Human fibrinogen γ-chain:

```
  1 Y V A T R D N C C I L D E R F G S Y C P T T C G I A D F L S T Y Q T K V D K D L
 41 Q S L E D I L H Q V E N+K T S E V K Q L I K A I Q L T Y N P D E S S K P N M I D
 81 A A T L K S R K M L E E I M K Y E A S I L T H D S S I R Y L Q E I Y N S N N Q K
121 I V N L K E K V A Q L E A Q C Q E P C K D T V Q I H D I T G K D C Q D I A N K G
161 A K Q S G L Y F I K P L K A N Q Q F L V Y C E I D G S G N G W T V F Q K R L D G
201 S V D F K K N W I Q Y K E G F G H L S P T G T T E F W L G N E K I H L I S T Q S
241 A I P Y A L R V E L E D W N G R T S T A D Y A M F K V G P E A D K Y R L T Y A Y
281 F A G G D A G D A F D G F D F G D D P S D K F F T S H N G M Q F S T W D N D N D
321 K F E G N C A E Q D G S G W W M N K C H A G H L N G V Y Y Q G G T Y S K A S T P
361 N G Y D N G I I W A T W K T R W Y S M K K T T M K I I P F N R L T I G E G Q Q H
401 H L G G A K Q A G D V
```

Fig. 3. (previous page) Amino acid sequences of human fibrinogen Aα-, Bβ- and γ-chains, sequences according to A. Henschen, F. Lottspeich, M. Kehl and C. Southan, Ann.N.Y.Acad.Sci, 408, 28-43 (1983); M.W. Rixon, W.-Y. Chan, E.W. Davie and D.W. Chung, Biochemistry 22, 3237-3244 (1983); D.W. Chung, B.G. Que, M.W. Rixon, M. Mace and E.W. Davie, Biochemistry 22, 3244-3250; D.W. Chung, W.-Y. Chan and E.W. Davie, Biochemistry 22, 3250-3256 (1983). Arrows and crosses indicate thrombin cleavage sites and carbohydrate side chains, respectively.

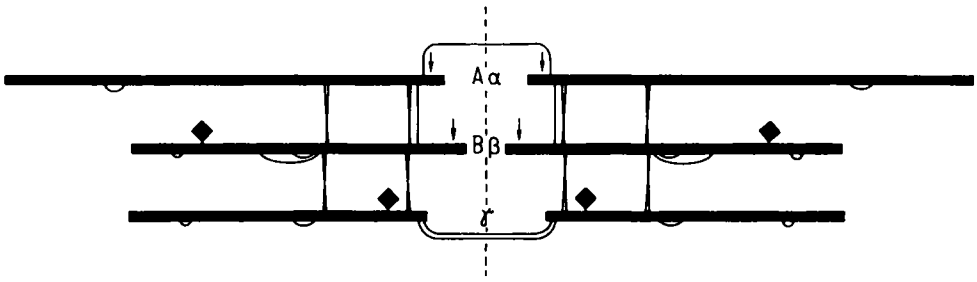

Fig. 4. Primary structure model of human fibrinogen, the chains being aligned according to homology, the N-termini with the thrombin cleavage sites (↓) in the middle; the thin lines represent disulfide bridges, the diamonds carbohydrate side chains and the broken line a center of symmetry.

II. GENE ANALYSIS

The Structure and Evolution of Fibrinogen: The Coiled Coil Region

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Introduction

The central event in the vertebrate coagulation system is the conversion of soluble fibrinogen into an insoluble fibrin gel. This is appropriately followed by the subsequent dissolution of the fibrin gel. A mechanistic explanation of how these processes occur depends on an understanding of fibrinogen's structure.

One approach to understanding structure-function relationships involves the comparison of functionally similar proteins such as fibrinogen between various species. The primary structure of human fibrinogen has been determined directly at the amino acid level (1, 2) and indirectly through nucleic acid sequencing of the appropriate mRNA's (3, 4, 5, 6). We have undertaken the structural analysis of the rat fibrinogen genes. The isolation of cDNA clones for all three rat genes has been carried out (13). In addition, a full-length cDNA for the rat gamma gene has been sequenced (7). Using cDNA as probes, phage clones from a rat

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genomic DNA library of all three rat fibrinogen chains have been isolated and mapped (8). Exons which code for the intermediate disulfide knot and the first half of the putative "coiled coil" region have been sequenced. Thus, the amino acid sequences for those regions of the rat fibrinogen chains can be surmised.

Using the primary sequence of human fibrinogen Doolittle et al. (11) built elaborate models of the coiled coil region of the human fibrinogen molecule. Using these models they sought to determine the potential for helix formation in the part of the structure bound by the two sets of cysteine rings found in each half of the fibrinogen monomer. When the "helical-net" approach of McLachlan et al. (12) was applied, a non-polar face could be seen for each of the chains. This led them to propose a parallel three-stranded rope that would begin with a coiled coil, would be interrupted in its central portion, and then would resume the coiled coil structure. The key to this configuration is the alternating pattern of the polar and non-polar amino acids that occur within the coiled region. The feasibility of such a structure was confirmed by the building of three dimensional models.

The comparison of the rat and human amino acid sequence in the region of the proposed "coiled coil" shows a large number of differences between the two species. However, the differences are such that the alternating arrangement of polar and non-polar amino acids in that region of fibrinogen molecule is preserved. Thus, the pattern Doolittle (11) found in the human molecule is almost certainly of structural significance. Moreover, the region that interrupts the coiled coil in the human is further interrupted in the rat fibrinogen molecule by the presence of three prolines in the rat gamma chain.

Materials and Methods

The appropriate Hind III DNA fragments were subcloned from Charon 4A phage isolates that have been described (8) into the plasmid pBR322. To determine the sequence of these DNA fragments the plasmid DNA was further subcloned into the M13 vectors mp-8 and mp-9.

The DNA sequence of M13 subclones was determined by the method of Sanger (9). A 15-mer from New England Biolabs was used for all reactions. However, whenever ambiguities were apparent a 15-mer (obtained from Bethesda Research Labs) having another sequence was used. Sequencing reactions were carried out with the Klenow fragment of DNA polymerase from several suppliers. Reactions were performed at 20°C or 37°C and were run on denaturing 6% acrylamide gels. Sequences were determined for both strands of DNA for 80% of the regions reported here. Sequences for the gamma region have been previously reported (7). Sequence analyses were carried out using the program of Queen and Korn (10) and microcomputer programs from the laboratories of Marshall Edgell and Clyde Hutchinson.

Results

The nucleic acid sequence of those exons that code for the intermediate disulfide knot and nearby amino acids is shown in Figure 1. The analysis of the rat genomic sequences revealed a splice junction corresponding to a position three amino acids proximal to the first cysteine of the intermediate disulfide knot. That exon ends approximately 60 amino acids downstream in all three genes. In Figure 1 the sequences have been aligned to show homology between these three exons. This required the insertion of spaces in the alpha and gamma sequences because the beta chain has an

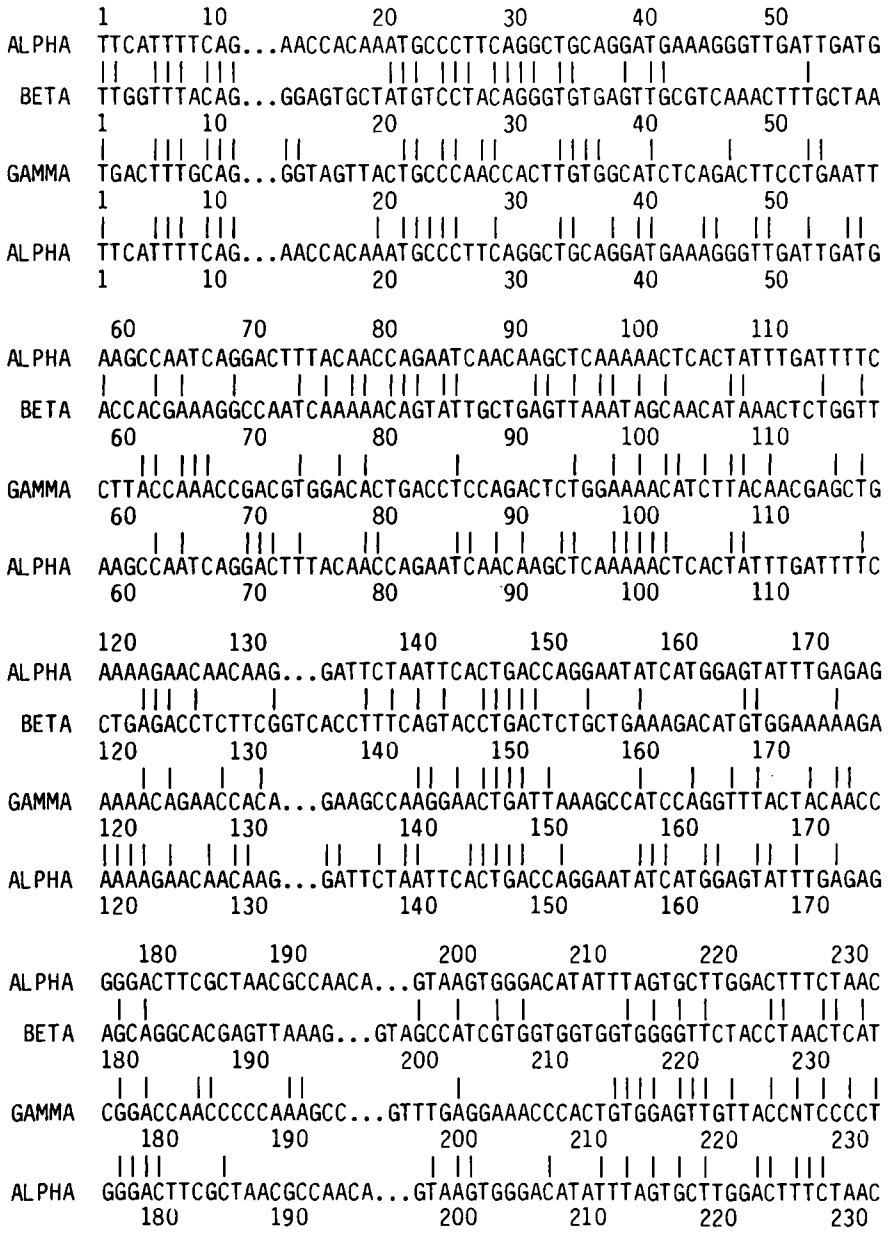

Figure 1. The genomic sequence of the rat fibrinogen exons coding for the intermediate disulfide knot and the first half of the "coiled coil" region. The sequences have been aligned optimally to show the maximum homology between the alpna, beta and gamma rat fibrinogen genes. The tickings flag homologous nucleotides.

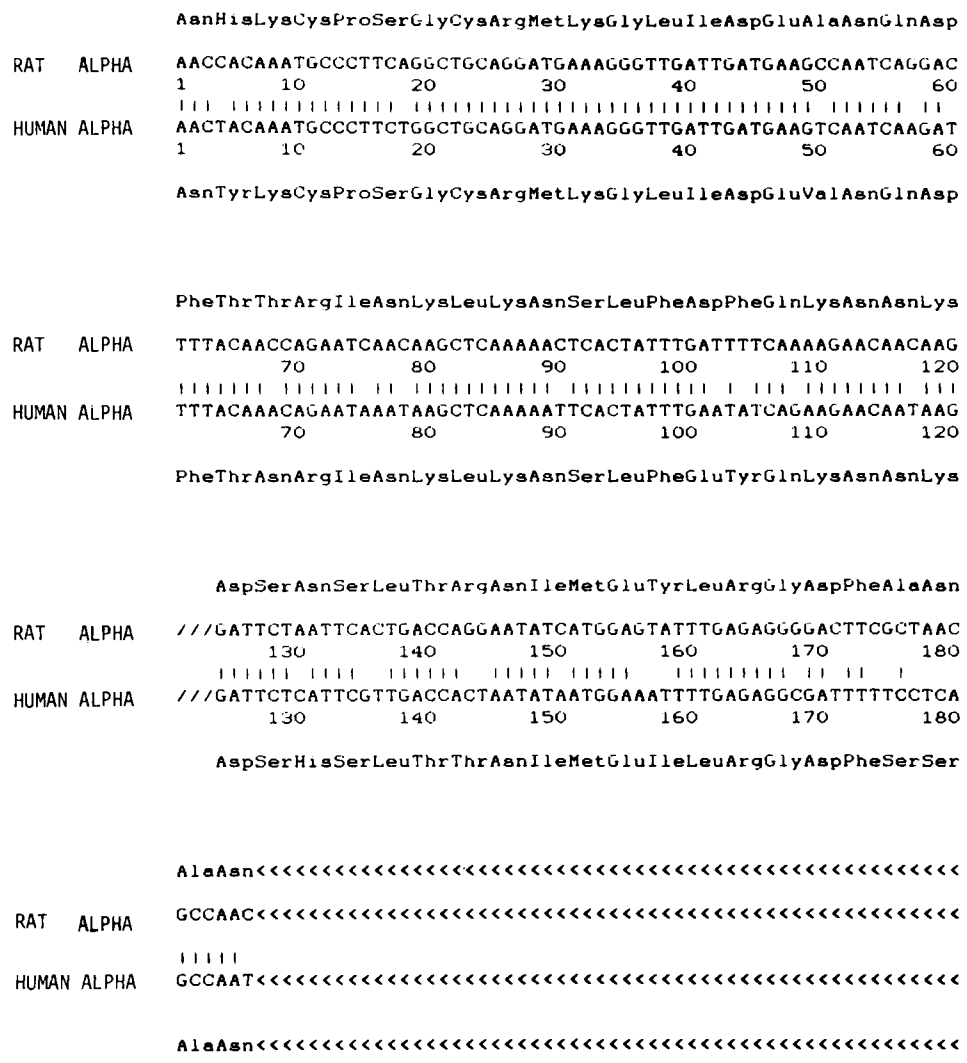

Figure 2. The nucleotide and amino acid sequences coding for the intermediate disulfide knot and the first half of the "coiled coil" region of the rat and human alpha fibrinogen chain (ref. 3, 4). The sequences have been aligned to show maximal homologies.

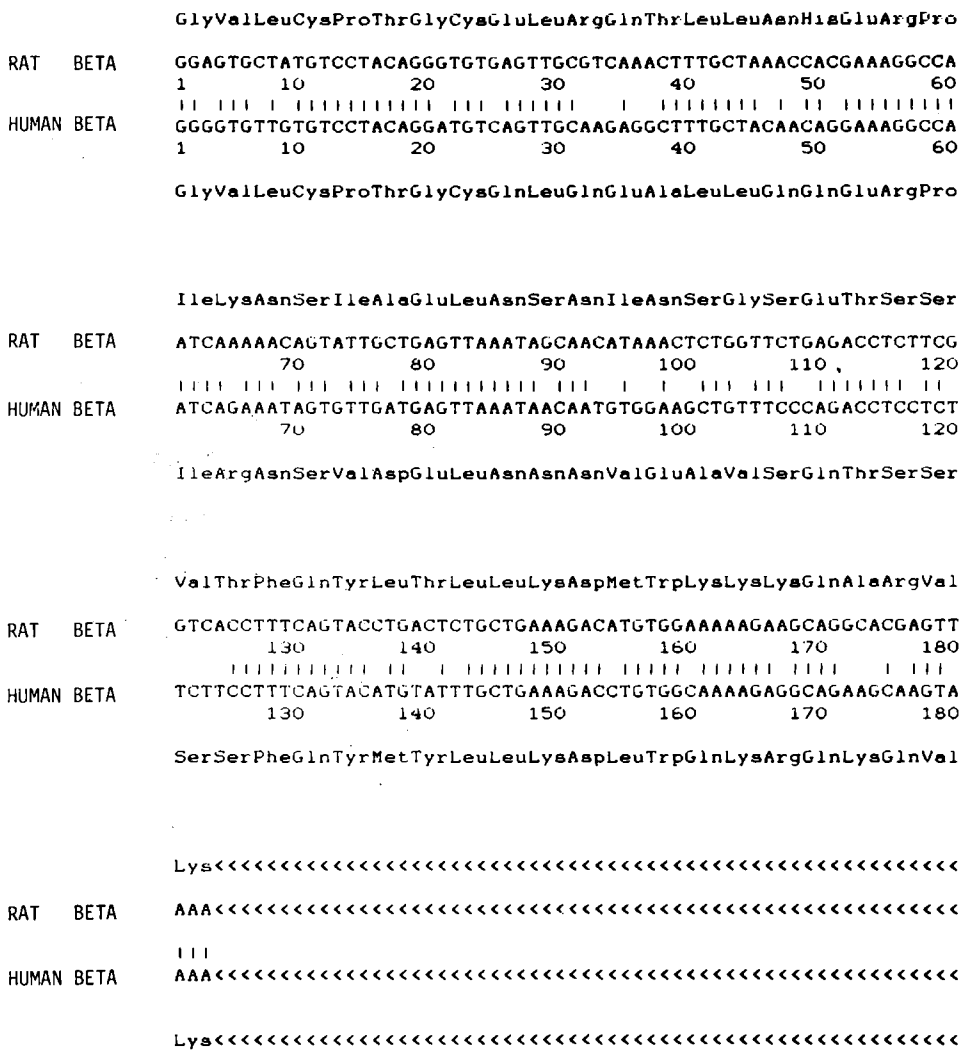

Figure 3. The nucleotide and amino acid sequences coding for the intermediate disulfide knot and the first half of the "coiled coil" region of the rat and human beta fibrinogen chain (ref. 5). The sequences have been aligned to show maximal homologies.

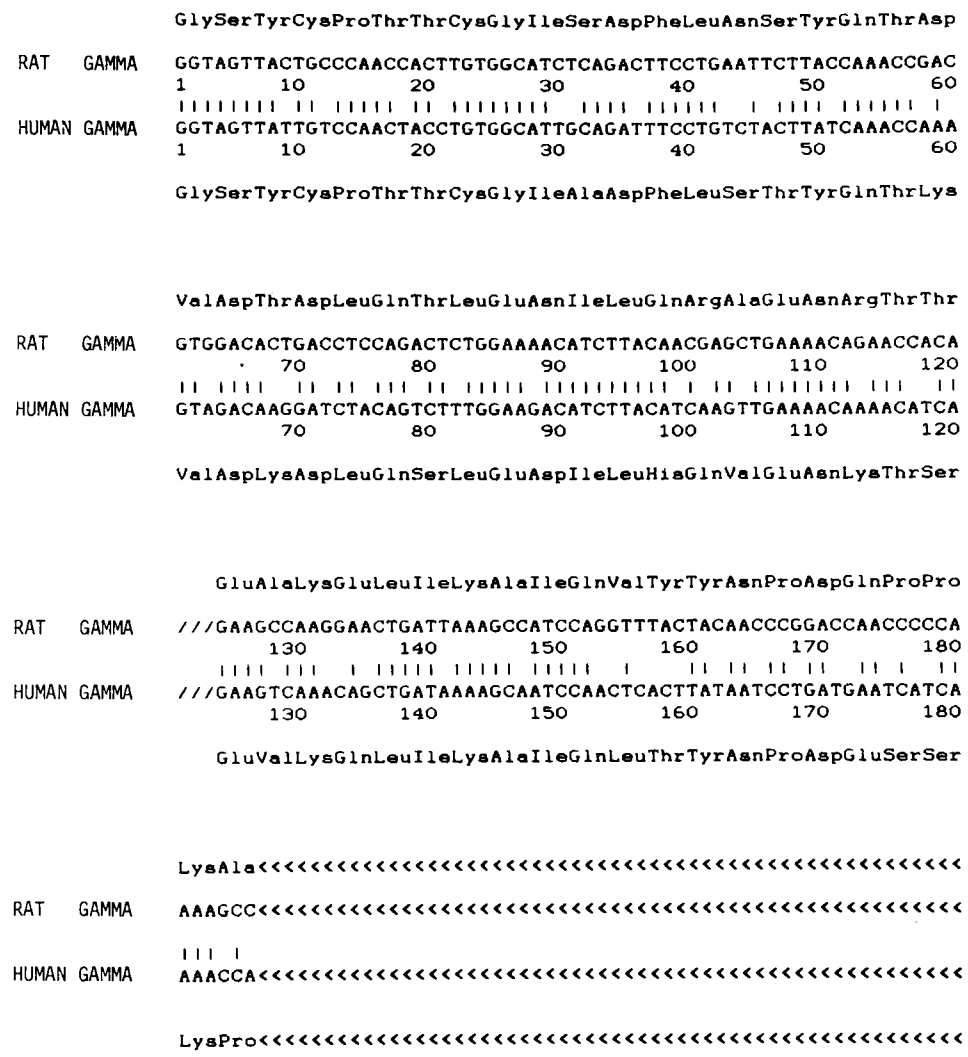

Figure 4. The nucleotide and amino acid sequences coding for the intermediate disulfide knot and the first half of the "coiled coil" region of the rat and human gamma fibrinogen chain (ref. 6). The sequences have been aligned to show maximal homologies.

amino acid whose counterpart is not seen in alpha or gamma. This is the only region of the fibrinogen genes where the same intron-exon arrangement occurs in all three chains (unpublished data). That is, other exons do not have homologous coding information to their counterparts. For instance, while the exon coding for the third disulfide knot in beta and gamma also contains the coding information for the second half of the coiled coil region, that is not the case for the alpha chain. Thus, this exon coding for the intermediate disulfide knot is the best conserved based on intron-exon structure. Yet one can readily see that there is little nucleic acid homology. There is 31% homology between alpha and beta, 31% between beta and gamma and 37% between gamma and alpha. This is only slightly greater than what would be expected by chance. However, there is 36% homology in the first position of codons, 46% in the second position but only 25% in the third or neutral position.

Figures 2, 3 and 4 show the inferred amino acid sequence of the intermediate disulfide knot in the rat compared with its human counterpart. An analysis of these differences are shown in Table 1.

TABLE 1

Summary of the differences between human and rat amino acid sequences within the exons coding for the first half of the coiled coil region. The apparent changes from rat to human are shown.

	Non-polar to non-polar	Polar to polar	Non-polar to polar	Polar to non-polar
alpna:	3 of 18	5 of 43	1	1
beta:	4 of 20	13 of 41	3	4
gamma:	3 of 20	14 of 41	2	1
total:	10 of 58	31 of 125	6	6

Discussion

The comparison of the rat and human fibrinogen sequence for that portion that includes the intermediate disulfide knot demonstrates the importance of certain portions of the primary sequence. One of the most noticeable features is the preservation of the Cys-Pro-Y-Z-Cys structure of the intermediate disulfide knot. The selective pressures to maintain the relative positions of the cysteine pairs and the proline is obvious. The important function of the cysteines to form interchain bridges is a simple explanation for their preservation. However, the function of the prolines is not as easy to appreciate. Is it possible that they function in a helix-altering capacity in order to assure that the cysteines have the opportunity to form the appropriate interchain bridges? Without the prolines between the cysteine pairs, the structure in the region of those cysteines might remain internalized as do the adjacent non-polar residues within the coiled coil and thus fail to establish interchain bridges.

Another feature that seems to be well preserved is the arrangement of polar and non-polar amino acid residues. It is remarkable that there are 52 amino acid differences between the human and rat fibrinogen molecules in this region and yet there is a striking conservation of the polar and non-polar amino acid composition. Relative to human fibrinogen the periodicity of non-polar and polar residues is well maintained in the rat fibrinogen, suggesting to us that this region of the fibrinogen gene is under little constraint to maintain any particular nucleic acid composition. Apparently the only constraint on the nucleic acids is that required to maintain the locations of the polar and non-polar residues. This is highlighted by the lack of conservation between the three genes in the third nucleotide position of

homologous genes versus the high degree of conservation (47% between all three genes in the rat) in the second codon position. The most likely explanation for this is the relative importance of the second nucleotide position in determining the polar versus non-polar nature of an amino acid residue. A thymidine (uridine in the coding mRNA) in the second codon position always codes for a non-polar amino acid. In the genetic code there are forty codons which use adenine, cytosine or guanine in the second codon position for polar amino acids yet only five codons use these bases in the second position to code for non-polar amino acids. Thus, one would predict that if functional constraints are limiting the evolution of the fibrinogen molecule that there would be certain biases in range of differences seen between different species. This appears to be the case. For instance, there are relatively few non-polar to non-polar amino acids substitutions shown here between the rat and human genes when compared to the number of polar to polar substitutions. Being teleological, one can see that fewer nucleic acid substitutions are tolerable to codons for non-polar residues than for polar ones. That appears to be because uridine is so frequently used in the second codon position for non-polar amino acids.

One question that has raised so much interest among those studying fibrinogen is the ancestral relationship of the fibrinogen chains to each other. It has been proposed that the fibrinogen genes may have arisen from a common ancestor 1000 to 1500 million years ago (1, 14). The natural rate of nucleic acid changes in the neutral positions of genes is too high to permit meaningful observations from the nucleic acid sequence data. For instance, the homology in the neutral codon position between the three rat chains shown in Figure 1 is only 25%, i.e., that which one could expect to occur by

chance alone. However, this must not be interpreted as evidence that the three genes are unrelated. Assuming that they did arise from a common ancestral precursor, there has been adequate time for natural mutations to decrease their homology to background levels. From the data we presented here, it would appear that similarities between the three genes may be either the result of a convergent form of evolution or from the snaring of common ancestors. The functional need for cysteine bridges and alpha helical structures may indeed be responsible for the similarities in alpha, beta and gamma fibrinogen genes.

References

1. Henschen, A., Lottspeich, F., Kehl, Southan, C.: Ann. N.Y. Acad. Sci. 408, 28-43 (1983).
2. Doolittle, R.F., Watt, K.W.K., Cottrell, B.A., Strong, D.D., Riley, M.: Nature 280, 464-468 (1979).
3. Kant, J.A., Lord, S.T., Crabtree, G.R.: Proc. Natl. Acad. Sci. USA 80, 3953-3957 (1983).
4. Rixon, M.W., Chan, W-Y., Davie, E.W., Chung, D.W.: Biochemistry 22, 3237-3244 (1983).
5. Chung, D.W., Que, B.G., Rixon, M.W., Mace, M., Davie, E.W.: Biochemistry 22, 3244-3250 (1983).
6. Chung, D.W., Chan, W-Y., Davie, E.W.: Biochemistry 22, 3250-3256 (1983).
7. Crabtree, G.R., Kant, J.A.: Cell 31, 159-166 (1982).
8. Kant, J.A., Crabtree, G.R.: J. Biol. Chem. 258, 4666-4667 (1983).
9. Sanger, F., Nicklen, S., Coulson, A.R.: Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977).
10. Queen, C.L., Korn, L.J.: Methods Enzymol. 65, 595-609 (1980).
11. Doolittle, R.F., Goldbaum, D.M., Doolittle, L.R.: J. Mol. Biol. 120, 311-325 (1978).
12. McLachlan, A.D., Stewart, M., Smillie, L.B.: J. Mol. Biol. 98, 281-291 (1975).

13. Crabtree, G.R., Kant, J.A., Fornance, A.J., Rauch, C.A., Fowlkes, D.M.: Ann. N.Y. Acad. Sci. 408, 457-466 (1983).

14. Doolittle, R.F.: Ann. N.Y. Acad. Sci. 408, 13-26 (1983).

ABSENCE OF GROSS DEFECT OF FIBRINOGEN GENES IN ONE PATIENT WITH CONGENITAL AFIBRINOGENEMIA

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INTRODUCTION

Fibrinogen is involved in qualitative and quantitative congenital disorders associated with bleeding disorders. Afibrinogenemia and hypofibrinogenemia usually describe total or partial quantitative deficiencies whereas dysfibrinogenemia usually represents patients with normal plasma level of functionaly deficient molecules. The hereditary pattern for dysfribrinogenemia and hypofibrinogenemia seems to be autosomal dominant whereas autosomal intermediate and recessive patterns have been reported for afibrinogenemia. The mechanisms involved in these inherited disorders are unknown. Various amino acid substitutions have been reported in dysfibrinogenemia and this may result most likely from point mutations. Hypofibrinogenemia has been associated with an abnormal intracisternal storage and may result from deficient hepatic secretion. Afibrinogenemia is a rare but not exceptional disease in which the plasma fibrinogen level is often undetectable with radioimmunometric assays. Since the first observation of Rabe and Salomon in 1920, more than 100 cases have been reported but the molecular basis for the lack of synthesis of fibrinogen in these patients is unknown. In the recent past years, considerable progress have been made on the molecular genetic of fibrinogen. The three chains of the molecule are synthesized under the direction of three different mRNAs, transcripted from three coodinated genes (1-4) linked into a portion of chromosome 4 with the following sequence 5'-γ - Aα - Bβ -3' (5-6).

Fibrinogen - Structural Variants and Interactions

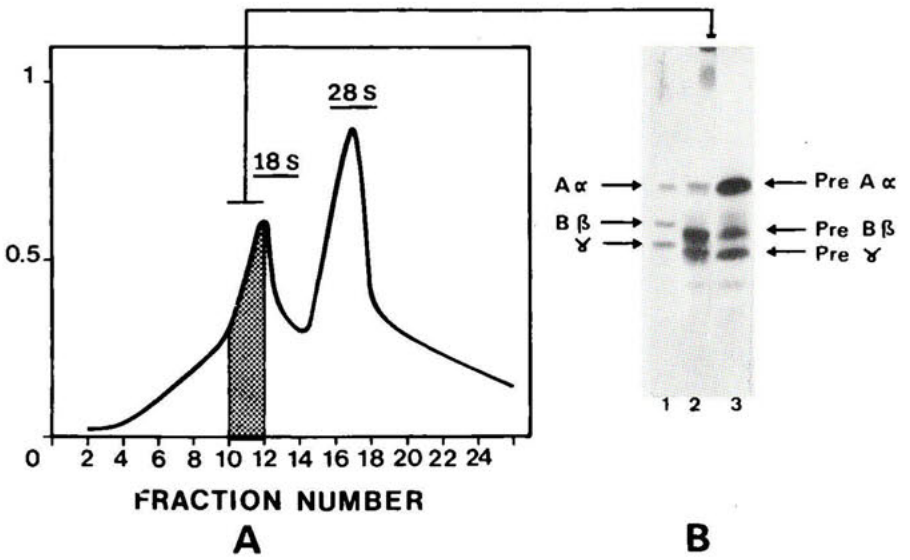

Fig. 1 Sucrose density gradient fractionation of human liver poly A^+ mRNA

Panel A : Sedimentation profile

Panel B : Fractions 10-12 were translated in a cell free system in the presence of ^{35}S-methionine. The synthesized material was immunopurified and analyzed on 5 to 20% polyacrylamide gels in the presence of 2% SDS and 2 % 2-mercaptoéthanol. Lane 1 :Standard (^{14}C)-labeled human fibrinogen. Lane 2 : Fibrinogen encoded by fractions 10-12. Lane 3 : Fibrinogen encoded by a total mRNA preparation.

With the use of recombinant DNA it is now feasible to analyse the structure and the expression of the fibrinogen genes in patients with afibrinogenemia and to examine wether this is due to a major deletion or insertion, an abnormal RNA processing and/or metabolism or a translational defect.
In our group we have isolated human Aα , Bβ and γ cDNAs and we have recently initiated a study of the DNA from different patients with afibrinogenemia. This paper describes a preliminary restriction endonuclease analysis of the DNA of one of these patients.

cDNA CLONES FOR Aα,Bβ AND γ CHAINS OF HUMAN FIBRINOGEN

Poly A^+ mRNA were isolated from a total human liver RNA preparation by chromatography on oligo dT-cellulose and fractionated by sucrose gradient sedimentation in the presence of 20. mM methylmercury hydroxide (Fig. 1A). Different fractions of this gradient were translated in a cell free reticulocyte lysate system and the neosynthesized material was analyzed on polyacrylamide gels after immunoaffinity purification (4-7). Those fractions containing RNAs encoding for the preAα , preBβ and preγ chains were selected (Fig. 1B) and reverse transcripted to make single strand ^{32}P-cDNA hybridization probes enriched in fibrinogen sequences. These probes were used to screen an adult human liver cDNA library (8-10). Different negative probes were used for screening. The high molecular weight fractions from the sucrose gradient (18-20 S) and a preparation of human muscle mRNA were also reverse transcripted to make negative ^{32}P-cDNA probes. In addition a rat albumin cDNA was used for evaluation of non specific hybridization. Twenty clones out of 7,000 hybridized with ss^{32}P-cDNA prepared with enriched preparation of fibrinogen mRNA. Of these isolates, three were recognized to have inserts with coding sequences for the Aα , Bβ and γ chains (Fig. 2). The specificity of these inserts were based on the capacity of the probes to select mRNAs of the correct size which translated to yield the predicted preforms of the Aα , Bβ and γ chains. Restriction mapping of these inserts and map fitting with known cDNA for human fibrinogen chains allowed the identification of the coding sequences (Table I).

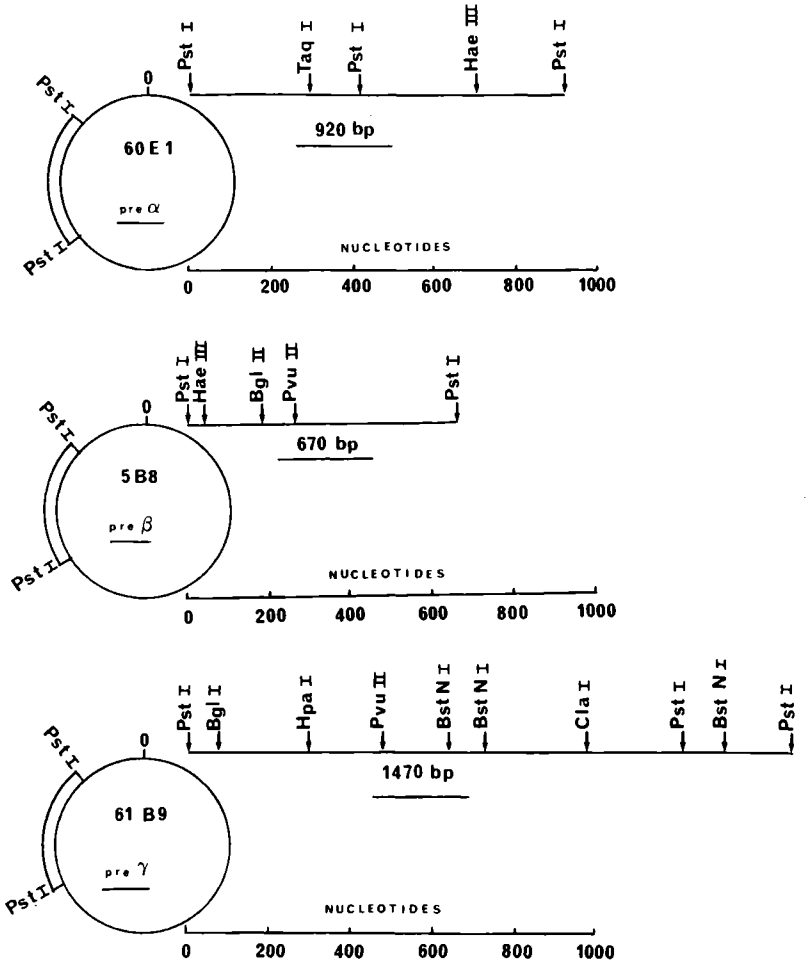

Fig. 2 Restriction maps of cDNA for Aα, Bβ and γ chains of human fibrinogen.

TABLE I

Coding sequences inserted in human Aα, Bβ and γ cDNA clones

Hybridization Probes	Chains	Sequence
60E1	pre Aα	GLY 404 to PRO 625
5B9	pre Bβ	ALA 277 to GLU 461
61B9	pre γ	MET -26 to VAL 411

ANALYSIS OF NORMAL AND AFIBRINOGENEMIC GENES

The recombinants were used as probes to analyse normal genomic DNA digested with various restriction enzymes. Single band patterns were observed, representing unique sequence consistent with the existence of a single copy of fibrinogen genes (Fig. 3). Analysis of the DNA from several normal individuals did not reveal restriction fragment polymorphism.

The DNA of one patient with congenital afibrinogenemia was analyzed with the same procedure. Absence of fibrinogen in the plasma of this patient has been documented for many years. Using a radioimmunoassay, the plasma level was less than 1.5 ug/ml. The DNA was isolated from peripheral white blood cells and digested with endonucleases. The pattern of digestion products obtained were indistiguishable from those obtained for normal DNA. These results indicated that fibrinogen genes, for Aα, Bβ and γ chains were present in the DNA of this patient who do not produce fibrinogen, and did not exhibit a major deletion. Further studies are now required to explain the lack of expression of the molecule in this patient. In the futur, genomic probes will be used to further characterize the gene defects present in afibrinogenemia.

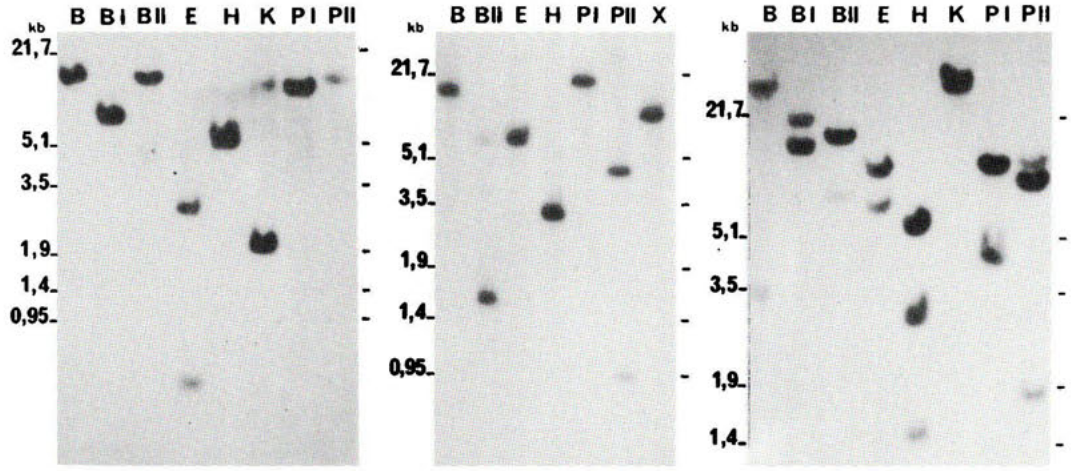

Fig.3 : Southern blot hybridization of human DNA digested with Bam HI (B), Bgl I (BI), Bgl II (BII), Eco RI (E), Hind III (H), Kpn I (K), Pst I (PI), Pvu II (PII), Xba I (X). The three filters were hybridized with nick translated purified inserts from Aα, Bβ and γ cDNAs.

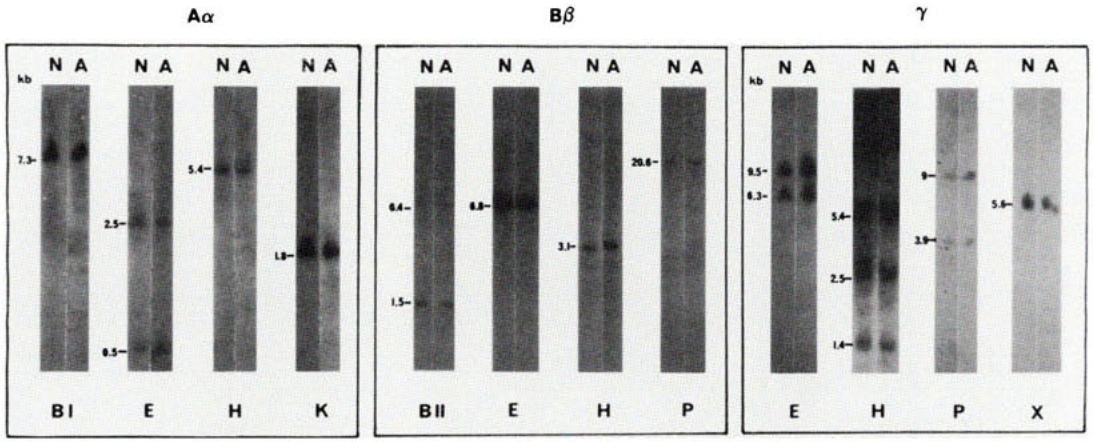

Fig.4 : Southern Blot analysis of normal (N) and afibrinogenemic (A) DNA digested with various restriction enzymes

References

1. Nickerson, J.M. and Fuller, G., Proc. Nat. Acad. Sci. USA, 78, 303-307, (1981).
2. Crabtree, G.R. and Kant, A. J., J. Biol. Chem. 256, 9718-9723, (1981).
3. Crabtree, G.R. and Kant, A. J., J. Biol. Chem, 257, 7277-7279, (1982).
4. Uzan, G., Besmond, C., Kahn A. and Marguerie, G., Biochemistry Int. 4, 271-274, (1982).
5. Kant, A.J. and Crabtree, G.R., J. Biol. Chem. 258, 4666-4667, (1983).
6. Henry, I., Uzan, G., Weil, D., Nicolas, H. Kaplan, J.C., Marguerie, G., Kahn, A., and Junien, C.: Am. J. Hum. Gen. 36, 760-768 (1984).
7. Kahn, A., Cottreau, D., Daegelen, D., and Dreyfus, J.C., Eur. J. Biochem. 116,7-12, (1981).
8. Sala-Trépat, J.M., Savage, M. and Bonner, J., Biochem. Biophys. Act. 519, 173-193, (1978).
9. Sala-Trépat, J.M., Sargent, T.D., Sell, S. and Bonner, J., Proc. Nat. Acad. Sci. USA, 76, 695-699, (1981)
10. Uzan, G., Courtois, G., Besmond, C., Frain, M., Sala-Trepat, J., Kahn, A. and Marguerie, G.,: Biochem. Biophys. Res. Comm. 120, 376-383 (1984).

III. FIBRINOGEN-FIBRIN CONVERSION