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血液病分子生物学

The Molecular Basis of BLOOD DISEASES

3rd Edition

人民卫生出版社
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THIRD EDITION

The Molecular Basis of Blood Diseases

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The Molecular Basis of Blood Diseases, 3rd edition
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ISBN:0-7216-7671-5

Authorized English language reprint edition published by the Proprietor.
Reprint ISBN: 981-4095-05-2

Harcourt Asia Pte Ltd.
583 Orchard Road, #09-01 Forum, Singapore 238884
Tel:(65) 7373593 Fax:(65) 7341874

图字：01-2001-2465

血液病分子生物学

编　　著： Stamatoyannopoulos 等
出版发行： 人民卫生出版社（中继线 67616688）
地　　址：（100078）北京市丰台区方庄芳群园 3 区 3 号楼
网　　址： http：//www. pmph. com
E－mail： pmph @ pmph. com
印　　刷： 三河市富华印刷包装有限公司
经　　销： 新华书店
开　　本： 889×1194　1/16　　**印张：** 65.5　**插页：** 5
字　　数： 2239 千字
版　　次： 2001 年 8 月第 1 版　2001 年 8 月第 1 版第 1 次印刷
印　　数： 00 001—3 000
标准书号： ISBN 7-117-04386-5/R·4387
定　　价： 260.00 元

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PREFACE

Since the second edition of this book was published in 1994, knowledge of the molecular basis of blood diseases has grown exponentially. This is reflected in the contents of the third edition of the book. Seven new chapters, on Stem Cell Biology, on Hematopoietic Growth Factors and Receptors, on Hematopoietic Transcriptional Factors, on Signal Transduction in the Regulation of Hematopoiesis, on Integrins in Hematology, on Paroxysmal Nocturnal Hemoglobinuria, and on Gene Therapy of Blood Diseases, have been added. All other chapters have been rewritten or extensively updated to reflect the explosion of knowledge. The first two editions established *The Molecular Basis of Blood Diseases* as a very useful resource for all individuals with an interest in hematology. We hope that the expanded and extensively updated third edition will be useful to a diverse audience, including established scientists, individuals engaged in teaching and the practice of hematology, postdoctoral fellows, and residents as well as medical and graduate students.

THE EDITORS

PREFACE to the Second Edition

Molecular biology has revolutionized hematology research. The first edition of this book, published in 1987, was among the first texts to examine the impact of molecular biology on disease mechanisms. In the intervening six years, the body of knowledge about proteins, cells and organisms gained by manipulation and characterization of DNA and RNA has grown exponentially. The challenge now is not only to understand disease mechanisms but also to apply this new knowledge to find more effective therapies.

Virtually all facets of hematology have now been subjected to study by molecular genetic techniques. Most inherited and many acquired diseases are now at least partially understood at the molecular level. Fundamental cellular mechanisms such as transcriptional regulation, signal transduction, antigen processing, and cell motility are coming to be understood. Our purpose with this second edition remains the same, namely to assemble this body of knowledge about gene structure, function, and organization and about disease mechanisms that form the basis for a molecular approach to hematology. The growth in information and our desire to provide a comprehensive exposition of principles has resulted in substantial increase in size of this second edition. Again we have relied on experts with broad perspective to write chapters related to their own areas of expertise.

The knowledge acquired by molecular techniques has broadened the scope of this edition of "The Molecular Basis of Blood Diseases." However, it, like the first edition, is not a textbook of hematology. No effort has been made to describe diseases for which molecular biological and sophisticated cell biological approaches have not yet yielded relevant information about disease mechanisms.

The book begins with a section, "Basic Concepts," that contains three chapters of broad relevance. An understanding of methods remains essential to comprehend the body of knowledge acquired by molecular techniques. Accordingly, Chapter 1 provides a general description of the methodology of molecular biology and serves as an introduction to gene structure and function. The mechanisms by which regulatory proteins interact with one another and with nucleic acids to regulate gene expression in determining patterns of cellular differentiation is addressed in Chapter 2. Blood-forming tissues are a dispersed hematopoietic organ that respond to microenvironmental influences including cytokines, negative regulators, and cytoadhesive molecules to achieve controlled production of red cells, lymphocytes, phagocytic cells including neutrophils, and platelets. Thereby the number of these elements remain fairly constant in circulating blood. Chapter 3 provides a comprehensive introduction to hematopoietic mechanisms.

Several chapters are included in the section on red cells. Effective treatment of sickle cell anemia and severe β thalassemia could be achieved if the fetal to adult switch during the perinatal period that initiates disease manifestations in affected individuals were reversed. Progress toward this goal achieved by application of molecular and cellular techniques provides a paradigm for understanding regulation of gene expression during development. Knowledge of the thalassemias, disorders reflecting deficient globin synthesis, illustrates the level of understanding about disease manifestations that can be achieved by consistent application of molecular methods. Sickle cell anemia, the first molecular disease for which the amino acid and nucleotide substitutions were known, remains challenging with respect to the pathophysiology of disease causing vaso-occlusive episodes. Since the first edition, there has been substantial progress in defining the structure of membrane proteins and surface antigens and mutations that lead to membrane dysfunction. Red cell enzyme defects, defined by classic biochemical techniques, have now come to be defined at the molecular level. New chapters on each of these topics have been included. Much progress has also been achieved in understanding how cells control iron uptake and storage to ensure availability for critical functions as described in the final chapter in this section.

Consideration of immunoglobulin and T-cell receptor gene rearrangements, lymphopoiesis, and the effector arm of the immune response has been expanded in Section III. These chapters are meant to provide a comprehensive account of important principles that have emerged as molecular knowledge about the immune system has grown. The function of phagocytic cells including endocytosis, the oxidative burst, and cell motility required much expanded consideration in the two chapters of Section IV.

Much progress has also been achieved in the study of hemostasis and its pathological counterpart, thrombosis, by application of molecular methods. The genes for the proteins involved in hemostasis and thrombosis have been characterized and mutations identified in individuals with deficiencies providing insights into protein structure and function. There is now a better understanding of the fibrinolytic mechanism and new therapies have been applied. Many new platelet functions have been characterized and these cellular fragments continue to provide novel insights into signaling mechanisms and cellular activation. The several chapters in Section V are designed to capture these new developments.

Neoplasms have come to be understood as acquired diseases with gene defects. Chromosome rearrangements create novel oncoproteins, and point mutations, gene amplification, or gene deletion either activate, increase or decrease critical cellular proteins. Each neoplastic cell has several mutations that interact in causing uncontrolled growth. Our approach, in Section VI, has been to emphasize important

principles with representative examples providing the framework to allow the interested reader to learn details through further reading.

Viruses manage to evade the immune system in establishing and maintaining infection, thereby creating disease by unique mechanisms. Section VII has been expanded to provide a comprehensive chapter on AIDS and a second chapter that covers other viruses that may invade and cause disease in both normal and immunocompromised individuals.

The size and weight of this book is one testimony to the impact of molecular biology on our understanding of the fundamental properties of the blood, bone marrow, and lymphoid organs and the elucidation of hematological diseases. What about therapy? Coagulation factor replacement, use of cytokines to stimulate hematopoiesis, and various fibrinolytic agents are current products of the molecular biological revolution. In the future, one hopes that pharmaceutical agents that target specific defective gene products or cellular functions will be discovered based on an appreciation of the molecular basis of blood diseases. The use of genes as investigative or therapeutic agents is already a clinical reality. Our decision not to cover this emerging area of research reflects the current status in which most research has focused on developing methodology and testing vectors in animal models. Undoubtedly future editions of this book will contain many examples of the successful use of gene therapy and other therapeutic approaches derived from molecular knowledge.

We hope that individuals of diverse backgrounds will find this book useful. For the serious student of hematology, whether medical student, resident or fellow, it will serve as a supplement to standard textbooks. Individuals engaged in the practice of teaching of hematology should find the book useful in learning and applying the principles of molecular biology in their discipline. The text should also be valuable to the graduate student, postdoctoral fellow, or established scientist with a working knowledge of molecular biology who desires to learn about the molecular basis of various blood diseases.

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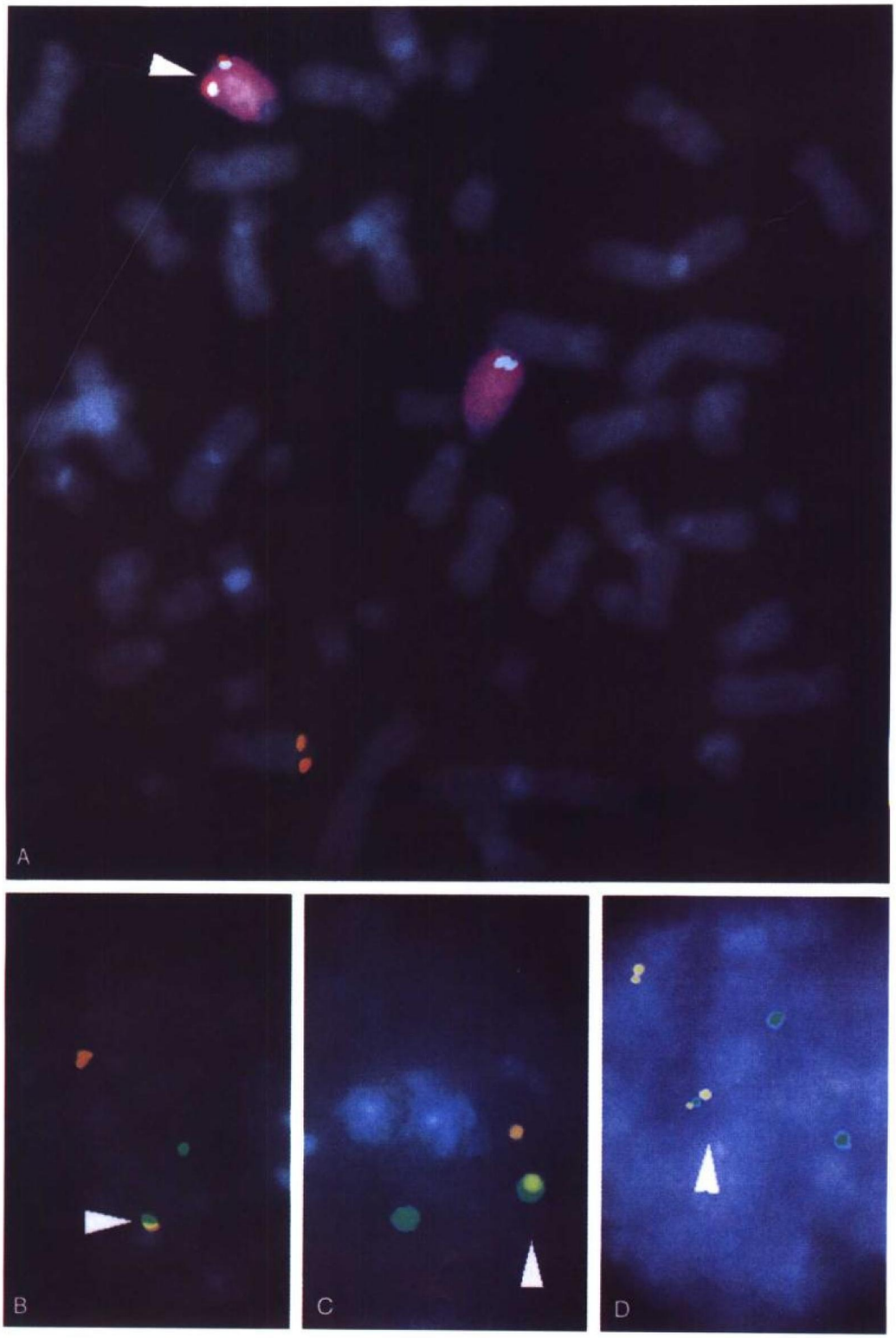

Plate 11–1. More sensitive identification of Ig translocations by metaphase or interphase fluorescent in situ hybridization (FISH) analyses. FISH of two myeloma cell lines was performed with a chromosome 14 painting probe (purple in *A*); a CH BAC probe (green in all panels) that includes Igα and Eα sequences from the centromeric end of the IgH locus (see Fig. 11–11); a c-*myc* plasmid probe (orange in *A* and *B*); a VH cosmid from the telomeric end of the VH locus (orange in *C*); and a pair of BAC probes that flank cyclin D1 and are separated by about 100 kb (orange in *D*). The first myeloma line (panels *A–C*) has a t(8;14) translocation. *A*, The arrow indicates juxtaposition of c-*myc* and CH on metaphase chromosome 14, plus a normal chromosome 14 with CH and a normal 8 with c-*myc*. *B*, The arrow indicates the t(8;14) translocation as manifested by close juxtaposition (red/yellow/green signal) of c-*myc* and CH in interphase nuclei. *C*, The split VH and CH signals demonstrate the presence of a translocation, with the arrow indicating the normal juxtaposition of CH and VH probes as an overlapping yellow/green signal. *D*, A second myeloma line shows CH sequences inserted between two sequences that flank the cyclin D1 oncogene, as indicated by the arrow. Molecular cloning showed that the CH sequences in *D* include Eα1 and other intervening sequences released during intrachromosomal switching from μ to ϵ in this tumor (see text and Fig. 11–11).[206, 212] The pictures were kindly provided by A. Gabrea and Y. Shou.

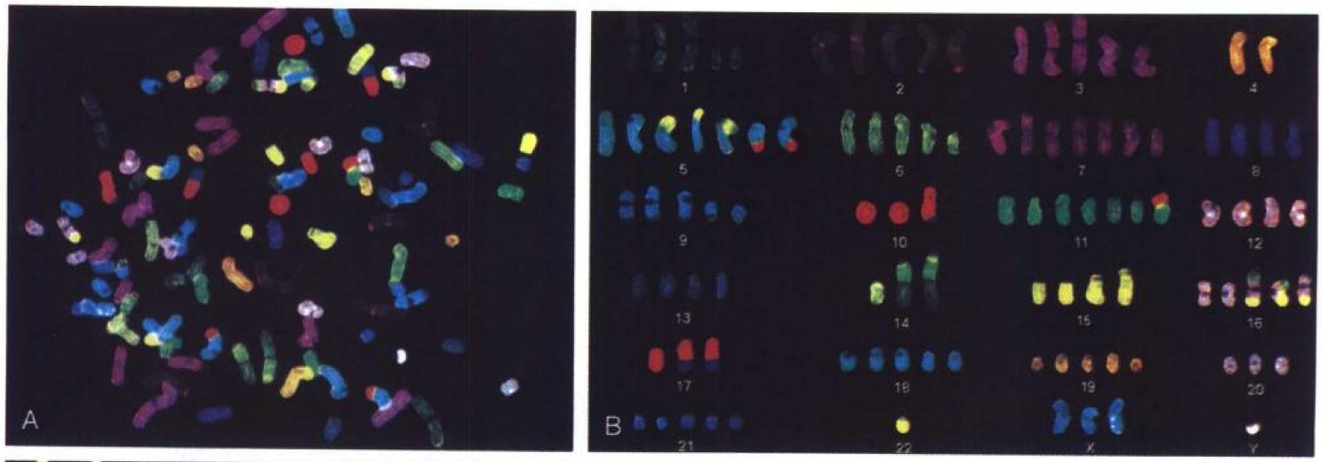

Plate 11–2. Spectral karyotyping (SKY) of a metaphase from a multiple myeloma cell line. *A*, Visualization of a metaphase spread with simultaneous identification of each distinct chromosome in different colors after hybridization with 24 combinatorially labeled chromosome painting probes. Chromosome painting probes were prepared in a two-step degenerate oligonucleotide primed (DOP) PCR of a human chromosome library, using combinations of different fluorochromes for labeling.[240] Spectral image acquisition and analysis were performed on a Leica DMRXA microscope equipped with the SD200 SpectraCube system and related software (Applied Spectral Imaging).

B, Ordered arrangement of the metaphase in *A*.

C, Karyotype of *B* shown in "classification" colors. A spectrum-based classification algorithm allows unambiguous identification of all pixels in the image that have the same or similar spectra. All pixels with the same spectrum are assigned the same classification color. The origin of all rearranged chromosomes was identified in this experiment. The numbers next to the aberrant chromosomes indicate the origin of translocated material. (These pictures and analysis were kindly provided by A. Roschke.)

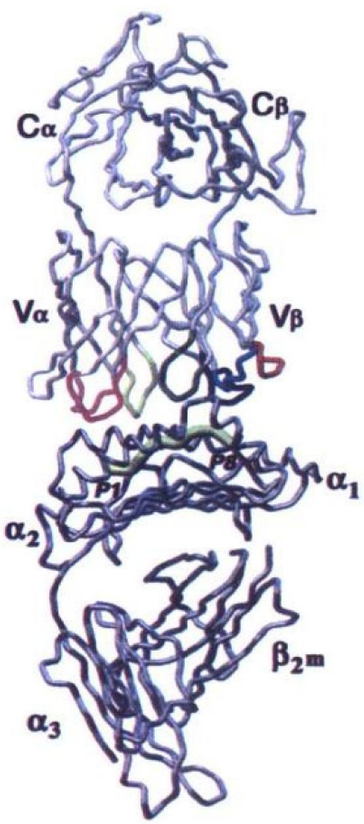

Plate 13–1. Structure of the T-cell receptor visualized by X-ray crystallography. Shown is a backbone rendering of the mouse 2C T-cell receptor binding to its cognate ligand, an ovalbumin-derived peptide presented by the H-2K^b class I gene. The T-cell receptor constant regions are at the top of the figure, viewed from a perspective perpendicular to the plane of the cell membrane, with the class I-peptide complex immediately juxtaposed. The hypervariable loops of the Vα and Vβ regions interact with both the peptide and the α1 and α2 regions of the class I molecule. Reproduced from [146] with permission of the publisher.

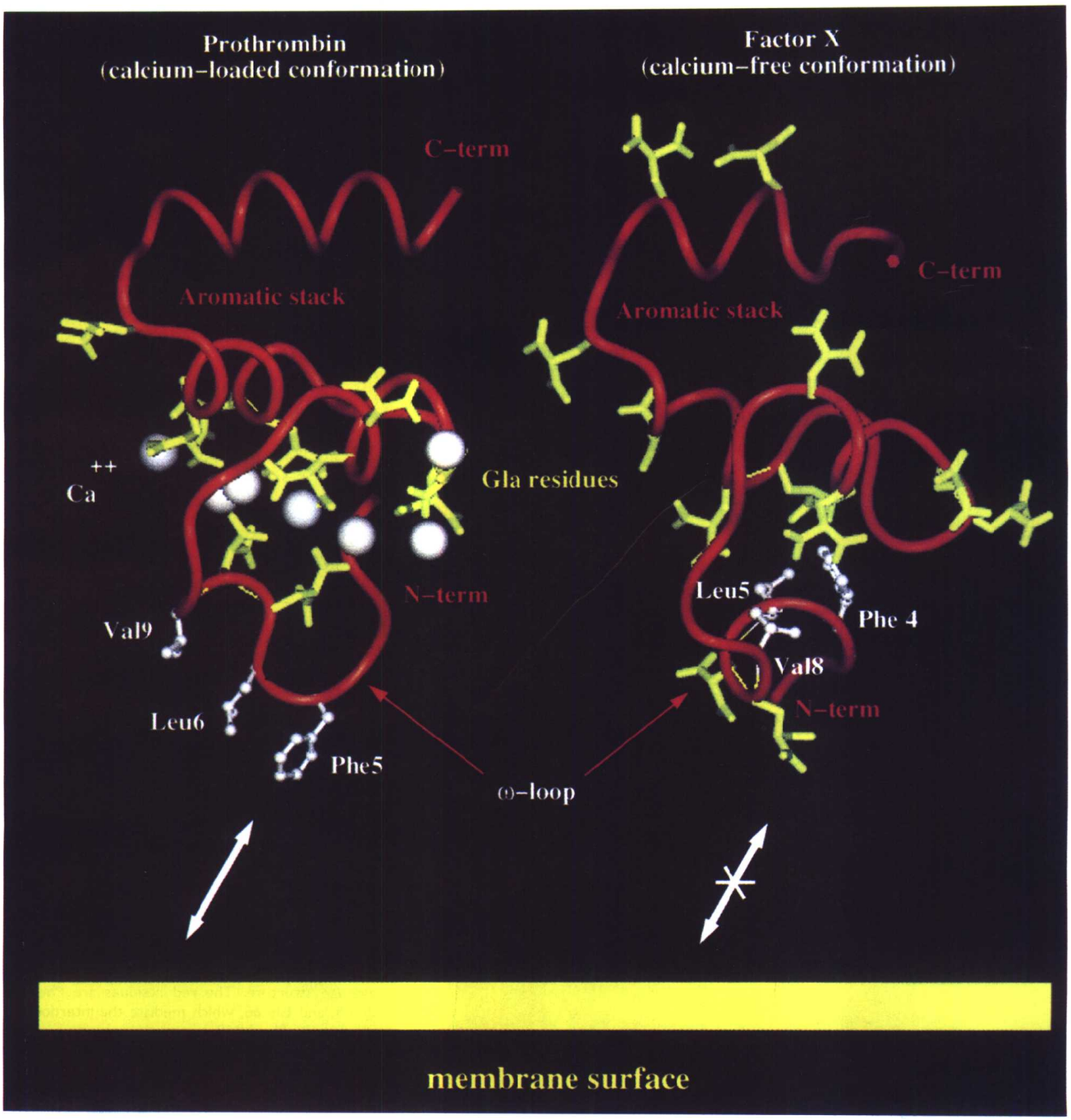

Plate 18–1. The NMR structure of the calcium-free form of the Gla module of factor X (bovine) is represented on the right. With the same orientation, the X-ray structure of the calcium-loaded form of the Gla module of prothrombin is displayed on the left side. Few side chains are shown to simplify the figure. The calcium ions are presented as spheres. Upon calcium binding, an important conformational change occurs mainly at the level of the N-terminal ω loop and results in the internalization of some negatively charged Gla residues (in yellow) and the exposure of three hydrophobic side chains (in light gray). The residues are Phe4, Leu5, and Val8 in factor X and in prothrombin the corresponding residues are 5, 6, and 9.

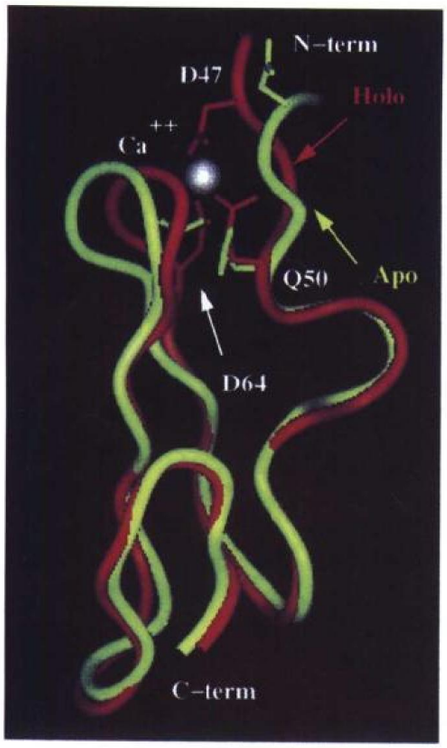

Plate 18–2. Schematic diagram showing the overall structure of the first EGF module of human factor IX. The X-ray structure of the holo form (red) of human factor IX[262] was superimposed onto the minimized average NMR structure of the same module in its apo form (yellow).[245] The key residues whose side chains are involved in calcium binding induce conformational changes within the N-terminal region of the module.

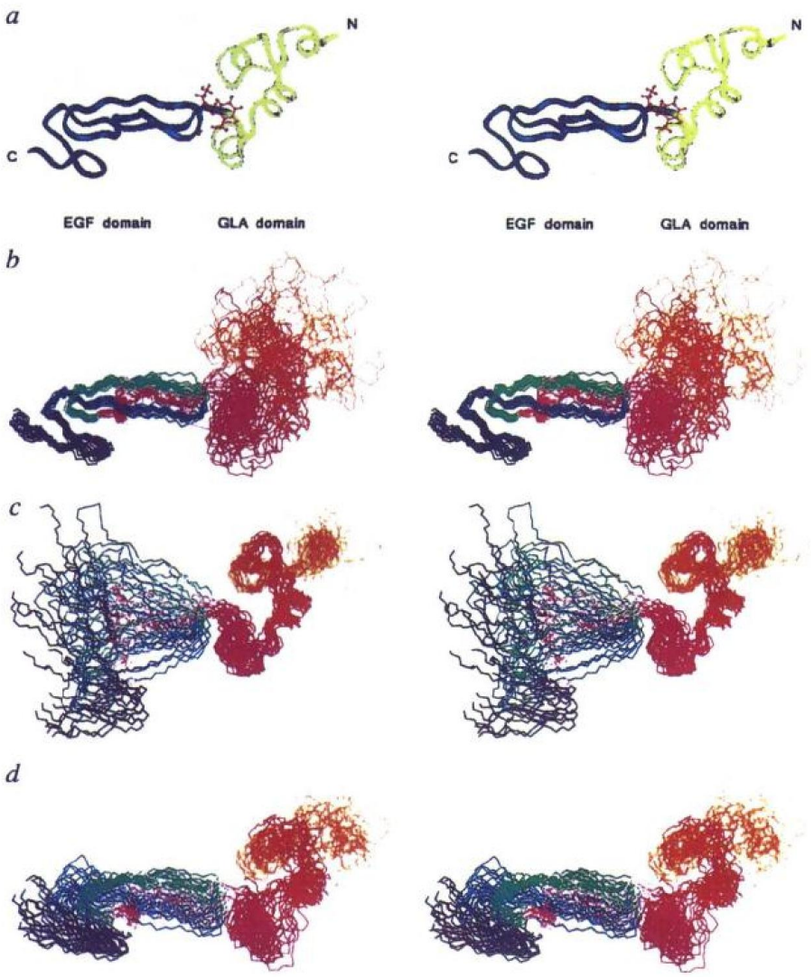

Plate 18–3. The GlaEGF module pair. *A*, Stereo ribbon drawing or the energy-minimized average structure. The red residues are Phe 40, Ile 65, and Gly 66, which mediate the interdomain contact. *B*, The NMR structures (obtained in the absence of calcium) were superimposed by minimizing the r.m.s.d. for the backbone atoms of residues 45-86 in the EGF module to the average structure. *C*, A family of NMR structures superimposed on the Gla module (residues 4-44). *D*, The family of NMR structures superimposed on the entire module pair (residues 4-86). Residues 1-19 are colored orange, 20-31 red, 32-44 magenta, 45-55 pink, 56-65 green, 66-75 blue, and 76-86 dark blue. Although the individual modules in the pair are well defined, their relative orientation is very poorly defined, indicating that they are joined by a flexible hinge region. The hinge is locked by binding of a single Ca^{2+} to the site in the EGF module.

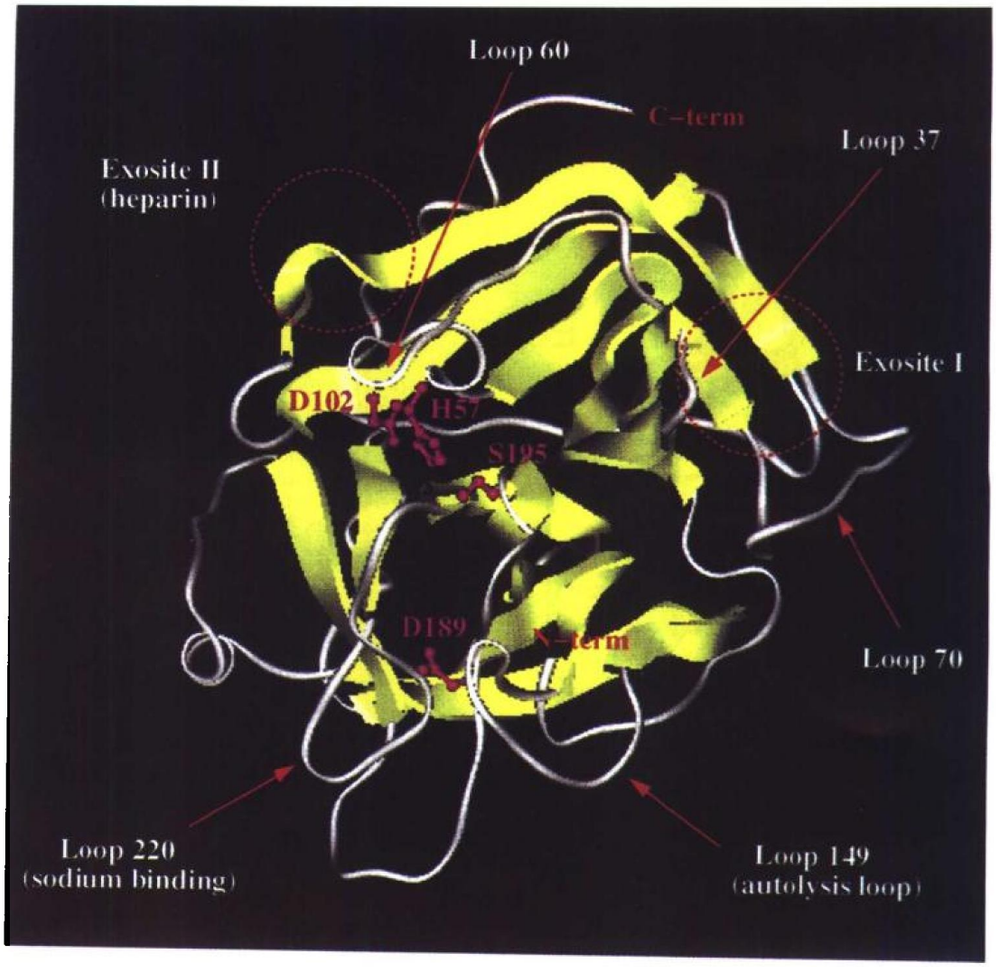

Plate 18–4. Thrombin structure. Richardson diagram of the B-chain of human thrombin with a view down the active side cleft.[37] Residues of the catalytic triad together with the residue at the bottom of the specificity pocket are shown in ball-and-stick representation (magenta). Important loop regions of the protein are noted. The anion-binding exosite I, important for the interaction with TM, fibrinogen, hirudin, and the thrombin receptors, is labeled. The anion-binding exosite II, known to interact with heparin, is also shown. The loop centered around residue 70 is homologous to the calcium-binding loop of trypsin, factors VII, IX, and X, and protein C.

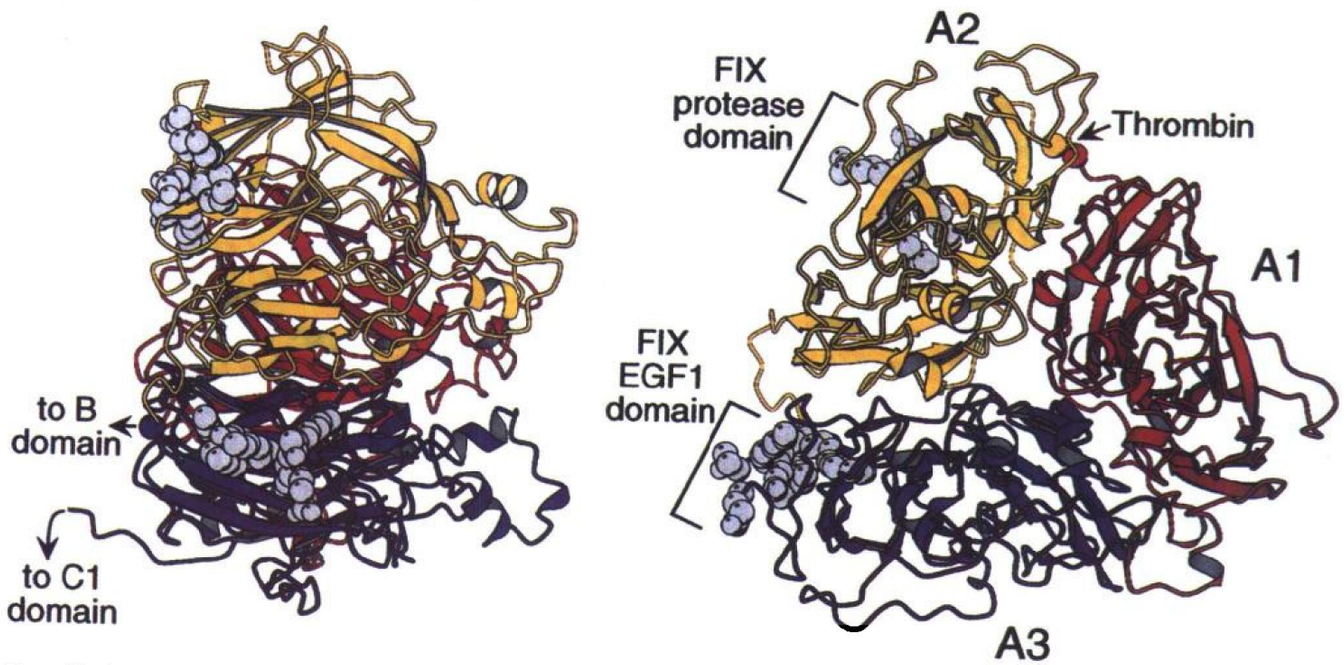

Plate 21–1. Molecular model of the factor VIII A domains. Two views are shown that differ by rotation of 90 degrees about the vertical axis. The right-hand view is looking down the pseudo threefold axis of symmetry. Domain A1 (red), A2 (yellow), and A3 (blue) are labeled. Helices are represented as coils, and the strands of β-sheets are represented as arrows. Side chains of residues proposed to interact with the factor IX protease domain and the first EGF-like domain are shown in space-filling (CPK) spheres. The termini of chains cleaved by thrombin also are indicated by spheres. In the left-hand view, termini that connect to domains not represented in the model are indicated. The carboxyl terminus of domain A1 continues into the B domain, and the carboxyl terminus of domain A3 continues into domain C1. The model was drawn with the program MOLSCRIPT[528] using the coordinates of Pemberton et al.[32]

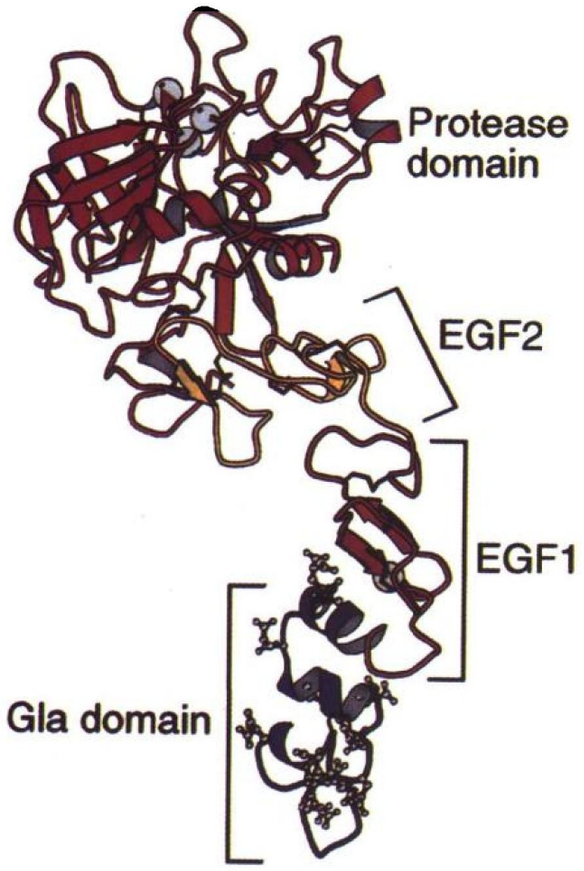

Plate 21–2. Three dimensional structure of porcine factor IXa. Domains of factor IXa are labeled: Gla domain (blue), the first EGF-like domain (EGF1, red), the second EGF-like domain (EFG2, yellow), and the serine protease domain (red). Gla residues are shown as ball and stick. The position of a β-hydroxyaspartic acid residue in EGF1 is shown as a gray sphere. Disulfide bonds are shown as black lines. The positions of α-carbons for the active site residues of the serine protease domain are shown as spheres. The domains shown in red (EGF1, Protease domain) appear to interact with specific sites in factor VIIIa. The model was drawn with the program MOLSCRIPT[528] using the coordinates of Brandstetter et al.[529]

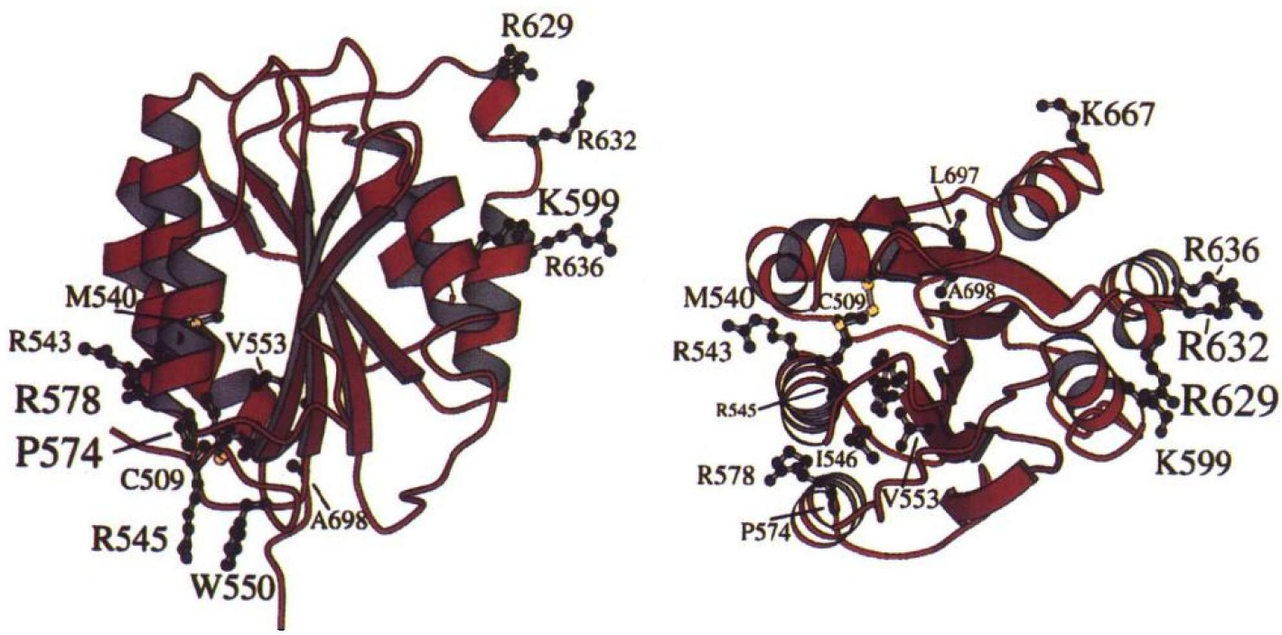

Plate 21–3. Structure of the VWF A1 domain. The two views differ by rotation of 90 degrees about the horizontal axis, so that the view on the right looks down on the top of the view at the left. Selected amino acids are shown by their side chains (ball and stick) and residue numbers (numbering from the amino terminus of the mature subunit). Mutagenesis studies suggest that Lys599 at the upper right interacts with platelet GPIbα, and that R636 and K667 interact with botrocetin; R629 and R632 may interact with either of these ligands. The residues clustered at the lower left (left panel) are mutated in VWD type 2B and may mark the location of a regulatory site that inhibits binding to GPIbα until VWF first interacts with connective tissue or certain soluble modulators. This figure was prepared with the program MOLSCRIPT[528] using the coordinates of Celikel et al.[411]

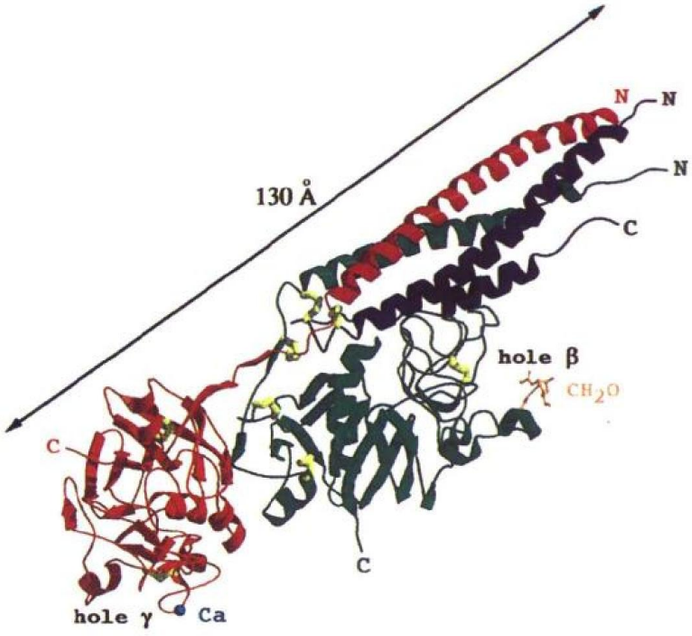

Plate 22–1. Ribbon representation of fragment D showing the region of coiled coils and the globular β and γ domains and the "holes" for binding "knobs." Blue, α chains; green, β chains; red, γ chains. Reproduced with permission from Spraggon, G., Everse, S. J., and Doolittle, R. F.: Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. Nature 389:455, 1997.

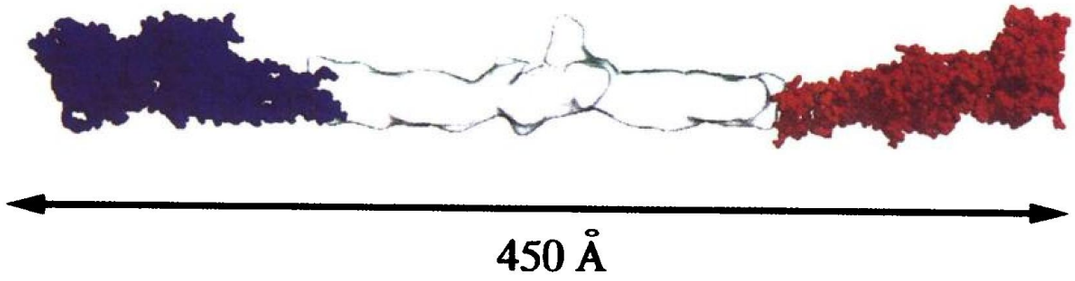

Plate 22–2. Reconstruction of fragment X based on structures of fragments D and double-D. Reproduced with permission from Spraggon, G., Everse, S. J., and Doolittle, R. F.: Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. Nature 389: 455, 1997.

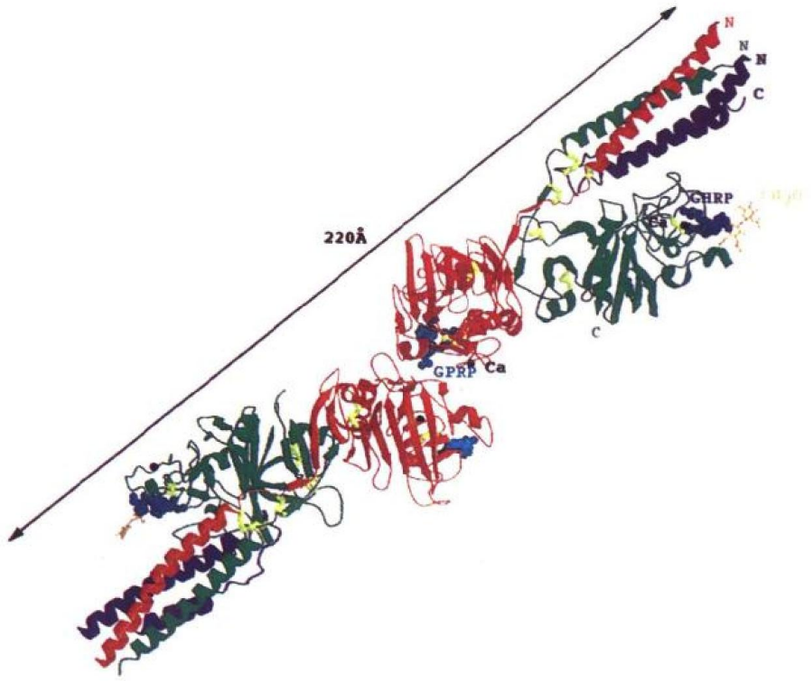

Plate 22–3. Ribbon structure of fragment double-D from cross-linked human fibrin with two different peptide ligands bound in different "holes." Blue, α chains; green, β chains; red, γ chains; GPRP, Gly-Pro-Arg-Pro-amide; GHRP, Gly-His-Arg-Pro-amide. Reprinted with permission from Everse, S. J., Spraggon, G., Veerapandian, L., Riley, M., and Doolittle, R. F.: Crystal Structure of Fragment Double-D with Two Different Bound Ligands. Biochemistry 37:8637, 1998.

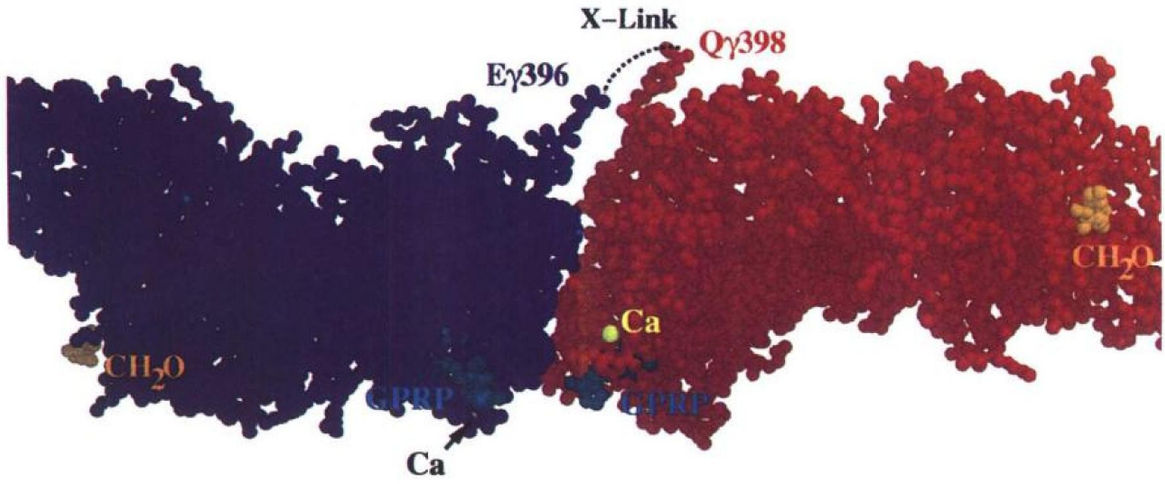

Plate 22–4. Structure of interacting D domains as determined from X-ray crystallography of fragment double-D. Reproduced with permission from Spraggon, G., Everse, S. J., and Doolittle, R. F.: Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. Nature 389:455, 1997.

Plate 22–5. Reconstruction of a protofibril as modeled from structures of fragment double-D with bound peptide ligands. Reproduced with permission from Spraggon, G., Everse, S. J., and Doolittle, R. F.: Crystal structures of fragment D from human fibrinogen and its crosslinked counterpart from fibrin. Nature 389:455, 1997.