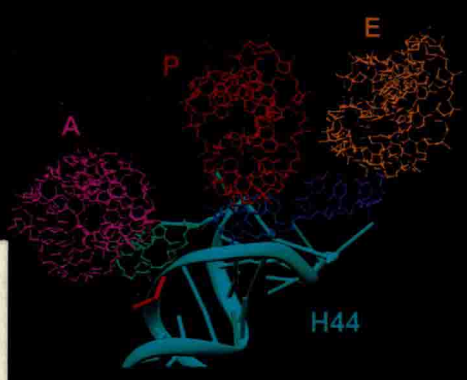
ANATOMY OF GENE REGULATION

A THREE-DIMENSIONAL STRUCTURAL ANALYSIS



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A Three-Dimensional Structural Analysis

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ANATOMY OF GENE REGULATION

A Three-Dimensional Structural Analysis

Determination of three-dimensional structures has revealed astonishing snapshots of molecules in action. No longer simple line drawings on a page, molecular structures can now be viewed in full-figured glory, often in color and even with interactive possibilities.

Anatomy of Gene Regulation is the first book to present the parts and processes of gene regulation at the three-dimensional level. Vivid structures of nucleic acids and their companion proteins are revealed in full-color, three-dimensional form. Beginning with a general introduction to three-dimensional structures, the book looks at the organization of the genome, the structure of DNA, DNA replication and transcription, splicing, protein synthesis, and ultimate protein death. The text discusses genetics and structural mechanics throughout. The concise and unique synthesis of information offers insight into gene regulation and into the development of methods to interfere with regulation at diseased states.

This textbook is appropriate for both undergraduate and graduate students in genetics, molecular biology, structural biology, and biochemistry courses.

Panagiotis A. Tsonis is Professor in the Department of Biology at the University of Dayton.

To my daughters
Isidora and Sol

Preface

What is in the structure? It is, of course, quality!

The determination of the three-dimensional (3-D) structure of DNA in 1953 heralded the beginning of molecular biology. At the same time, we saw one of the first examples of how the 3-D structure of a biomolecule reveals its function. The 3-D structure of DNA immediately suggested how the genetic information is passed to the progeny. Eventually, the discovery of DNA structure led to the understanding of how genetic information accounts for the final product, which is protein synthesis. For the past 40 years, research in molecular biology has led to the identification of a cascade in gene regulation from its packing into chromosomes to transcription, splicing, modifications, protein synthesis, and, finally, the death of proteins. Eventually, knowledge of the mechanisms involved in these events led to manipulation of genes, recombinant DNA, and cloning technology, all of which helped us grasp the function of genes and their role in the study of differentiation, development, and diseases.

As the major players at all the different levels of gene regulation were discovered, it became apparent that the final mechanisms will be best revealed when we can observe the action of enzymes and genes at the 3-D level. Information gathered by biochemical and molecular experiments could identify the function of an enzyme, say the role of DNA polymerase in replication or the role of helicase in unwinding DNA. However, only by observing the process at the 3-D level can we visualize and fathom the mechanism by which the enzyme possesses such activity. In fact, we can visualize enzyme action not only at the molecular level but also at the atomic level.

All this was possible because of the development of technology to determine the 3-D structure of biological molecules. In this sense, molecular biology has given rise to atomic biology where interactions between

biomolecules and mechanisms can be resolved at the atomic level. These techniques have become better over the years and undoubtedly will become even better. So far, the determination of 3-D structures has revealed astonishing snapshots of molecules in action and has made secrets known. We are able now to virtually study the anatomy of molecular events.

In this book, I present the story of gene regulation with emphasis at the 3-D level, thus the title "Anatomy of Gene Regulation." Like surgeons (molecular surgeons), we can now open the nucleus and other organelles and, with extraordinary glasses, see the mechanisms unfolding at the atomic level. I have elected to begin our discussion with the packing (organization) of DNA in the nucleus and then to explore DNA replication, transcription, and splicing, followed by RNA modification. Next, we will follow the path of mRNA to the cytoplasm and its decoding during protein synthesis. The final chapter will consider the birth and death of proteins, which is the end of the regulation process.

Why did I undertake such a task? Even though I am not a structural biologist, I have been teaching molecular biology for nearly 15 years. Throughout my teaching career, I realized that most of the molecular biology textbooks were incredibly massive, full of more information than can be covered in one semester. Furthermore, textbooks in different disciplines overlap considerably. Most textbooks on microbiology, genetics, molecular or cellular biology, and even developmental biology contain chapters on the basic aspects of gene regulation. Students, therefore, are exposed to the same contents thoughout their education. I believe that, at least in the study of molecular biology, some changes are warranted. Consequently, I slowly started incorporating the 3-D aspects. I soon realized that my course was becoming more unique and exciting to the students. Finally, I decided that a molecular biology course that emphasized 3-D aspects must be beneficial to both undergraduate and graduate students. Determining the 3-D structure of a mechanism is the ultimate level that we should go to if we are to understand the mechanism. Therefore, all disciplines dealing with cellular and molecular events will eventually need to study them at the 3-D level.

Producing this text was very challenging. For every structure presented in the book, I read the corresponding paper(s) very carefully and extracted only the information that would produce a sequence that flows well and is accurate. Therefore, my book is by design short and concise. It focuses on the 3-D aspects of gene regulation only. However, it does not leave out information pertinent to molecular biology when structures are not involved. I intend to focus on the 3-D aspects of gene regulation and not to overwhelm the reader with details that can be found in many other general textbooks and courses. Each chapter is preceded by a section, which I call primer. This section outlines the general plan of the sequence that I follow

in the chapter. It should help keep the reader focused on the main aim of the text.

Even a book on 3-D structures can be overwhelming. For example, the structure of many different DNA polymerases must be solved. Likewise, we must know the 3-D structure of numerous transcriptional factors. Obviously, reporting all of them in a book would be tedious for the reader. Therefore, I have filtered the information and concentrated on a series of structures that best represent the desired style of the book and its aim, which is to acquaint the readers with the 3-D aspects of gene regulation. At the same time, I have tried not to compromise quality and accuracy. The structures are presented in different ways using different models. This technique is deliberate because some aspects of a 3-D structure are better depicted by one model than by others.

Such a synthesis has not been attempted before, so I am sure that there will be supporters and critics. I do count on both to provide me with valuable comments on this project so that can improve it in the future.

Finally, I am grateful to numerous colleagues who have furnished me with the figures that are included in the book. Without their help and co-operation, my book would have been impossible to produce. The reference list is not extensive and is concentrated mainly on the papers that deal with the 3-D structures. General references on standard molecular biology can be found in several excellent textbooks, which are cited in the reference section.

CREDITS

Several databases, which have cataloged available three-dimensional structures of proteins, were a big help. Throughout the book numerous images have been used from these databases. These databases follow:

Berman, H. M., Olson, W. K., Beveridge, D. L., Westbrook, J., Gelbin, A., Demesy, T., Hsieh, S-H., Srinivasan, A. R., and Schneider, B. (1992). The nucleic acid database: A comprehensive relational database of three-dimensional structures of nucleic acids (NDB). *Biophys. J.* 63: 751–9. http://ndbserver.rutgers.edu/NDB/NDBATLAS/

Berman, H. M., Westbrook, J., Zeng, Z., Gilliland, G., Bhat, T. N., Weissing, H., Shindyalov, I. N., and Bourne, P. E. (2000). The Protein Data Bank (PDB). Nucleic Acids Res. 28: 235–42. www.rcsb.org/pdb

Reichert, J., Jabs, P., Slickers, J., and Suhnel, J. (2000). The IMB Jena Image Library of Biological Macromolecules. Nucleic Acids Res. 28: 246–9. www.imb-jena.de

Contents

CHAPTER ONE

A General Introduction to 3-D Structures

PRIMER The three-dimensional structure of nucleic acids and proteins as it pertains to the mechanisms involved in gene regulation is the major focus of this book. Therefore, the reader will encounter many 3-D structures. The first chapter of the book presents the very basic ideas behind the three-dimensional aspects of biomolecules. The first part deals with the techniques used to determine 3-D structures. The presentation is virtually for the layperson. Then the basic structural elements found in proteins are examined. Having done this, we examine a particular 3-D structure (that includes both DNA and protein) presented with different modeling. This exercise will help you to become familiar with the different ways that scientists present their 3-D structures. We use different models because one aspect of structure and function can be better represented with one model, whereas another aspect is more suited to a different model.

This book deals with the three-dimensional aspects of gene regulation. The reader will encounter numerous three-dimensional structures, but this should not scare anybody away. Unfamiliar readers might think that interpreting these structures is difficult, but this is not true. All we need is a basic introduction into the three-dimensional aspects of proteins and nucleic acids and the way that it can be represented. The basic 3-D structure of a protein can be reduced to two elements: the alpha helix and the beta strand (and loops that connect them). The complicated 3-D structure of a protein is a combination of several of these elements. Also, depending on the presentation, the alpha helix or the beta strand might be shown with different styles. To get started, let us review the main elements of the 3-D structures, the different representation

styles, and the basic methods used in the determination of the three-dimensional structures.

Two methods are generally used to determine the 3-D structure of a biomolecule (nucleic acid or protein). One method is Nuclear Magnetic Resonance (NMR) spectroscopy, and the other is X-ray diffraction (or X-ray crystallography). NMR uses properties of the atomic nuclei to determine how closely they are positioned. The so-called nuclear Overhauser effect (NOE) is a nuclear relaxation effect. This intensity is a measure of the distance between two nuclei that are close together. The two nuclei might be far apart in the primary sequence, but they could be close in 3-D because the protein is folded. Gathering data from all atoms enables the researcher to create a 3-D model of the molecule under investigation. NOE(s) are detected by NOE spectroscopy (NOESY) NMR experiments. The intensity of NOESY determines the actual distance between two nuclei. A strong intensity indicates that the two nuclei are 3 Å apart, a medium intensity measures less than 4 Å, and weak intensity is less than 6 Å. Because structure determination by NMR is in solution, ends or loops of proteins, which are flexible, are sometimes not solved well. For this, more than 20 calculated structures should be received and superimposed. At this point, we should be able to see the regions that are not defined well. Finally, based on all calculated structures, an average structural model can be produced. These superimposed structures appear throughout the text. One limitation of this method is that it can resolve structures of small proteins (about 30 kDa). However, a few proteins of about 50 kDa have also been solved, and future developments might push these limits. Also, because NMR determines structures in solution, the protein should be stable in solution.

The other method, using X-ray diffraction, can be applied to large molecules or even complexes of them. When using X-ray diffraction, the protein must be crystallized. The crystal is then exposed to X-rays, and a picture is received on a film where the diffracted light from the crystal produces patterns, depending on the 3-D structure of the protein. For example, the celebrated 3-D structure of DNA, which is a periodic pattern, produced spots on the film, which were symmetrically arranged. This symmetry led Watson and Crick to deduce that the DNA must have two periodicities, one from base to base and the other every helical turn (nearly every 10 bases; see Chapter 2). Obviously, most complicated 3-D structures, such as the ones found in proteins, would produce a more elaborate pattern on the film, but algorithms and techniques have been developed to put these patterns into a 3-D structure. X-ray diffraction would provide very clear 3-D solutions and does not have the limitations with the flexible regions as in NMR. The only limitation is that not all proteins can be crystallized efficiently. When the same structure has been solved with both NMR and X-ray diffraction, the results usually match very well, indicating that both methods are quite reliable.

Let us now familiarize ourselves with the basic structures in a protein. As noted earlier, the primary amino acid sequence can assume either a helical or a

beta strand conformation. Some amino acids are more likely than others to be in an alpha helix, and the same is true for amino acids found in beta strands. First, we will examine the basic structure of an amino acid and the peptide bond. All amino acids have a central carbon, C_α, to which a hydrogen atom, NH_2 (amino group), and COOH (carboxyl group) are attached. What discriminates the 20 different amino acids is the side chain, R, which is attached to the central carbon atom (Figure 1.1A). Amino acids are joined via the peptide bond to create polypeptides (Figure 1.1B). When amino acids are arranged in an alpha helix, there is hydrogen bonding between the C=O of a residue and

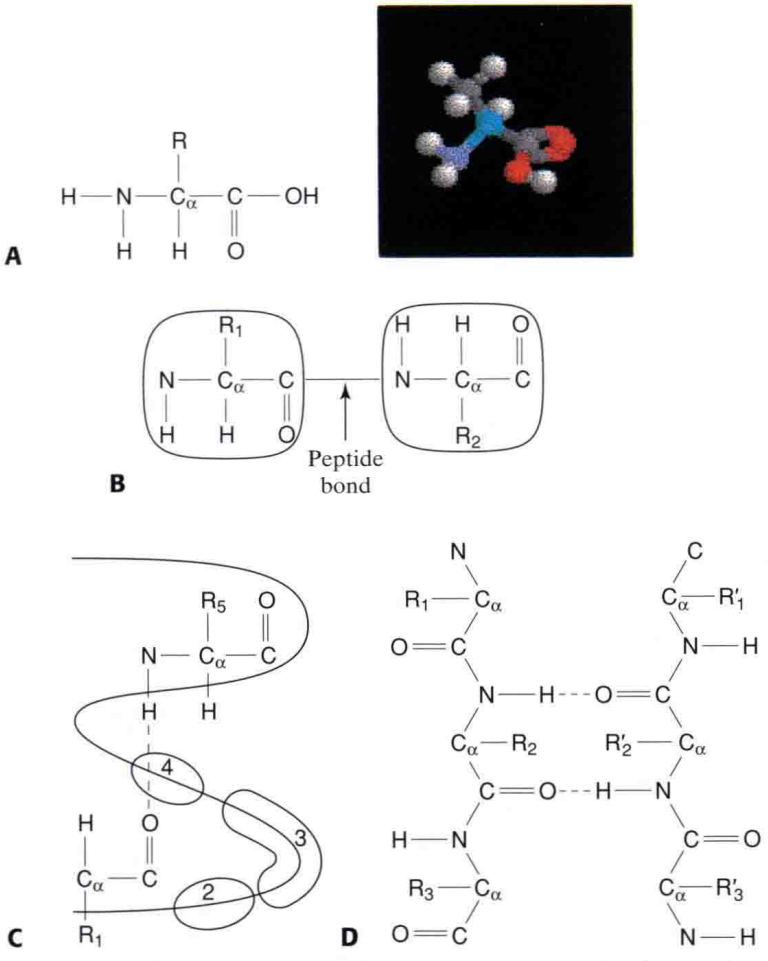

Figure 1.1. A: The basic chemical structure of an amino acid, indicating the standard H, NH_2, and COOH groups. R is the side chain that can vary in different amino acids. In the ball-and-stick representation, R is a CH_3 group and the amino acid is alanine. C is gray with the C_α cyan, N is blue, oxygen is red, and H is white. **B:** A dipeptide showing the creation of the peptide bond. **C:** An illustration of alpha helix. Note that residue 1 and residue 5 interact via hydrogen bonding (dashed line) using their C=O and NH groups, respectively. **D:** Two antiparallel beta strands creating a beta sheet via hydrogen bonding (dashed line) using an NH group from one strand and a C=O group from another. From F. R. Gorga, Protein Data Bank (PDB), Nucleic Acids Res. 28: 235–42.

the NH of another residue four positions away. In other words, there would be hydrogen bonds between residue 1 and 5, 2 and 6, and so on (Figure 1.1C). The alpha helix has 3.6 residues per turn, but variations exist with hydrogen bonds to residue $n + 5$ (pi helix) or $n + 3$ (3_{10} helix). Most of these helices are found at the end of alpha helices. The alpha helices are usually depicted as ribbons or cylinders in the 3-D structure of a protein.

The beta strand, and the resulting beta sheets from their interaction, is the second major element found in proteins. A beta strand contains 5 to 10 amino acids, which are in almost fully extended conformation. Interactions with adjacent beta strands can form a beta sheet. These interactions involve hydrogen bonding between the C=O of one strand and the NH group of another (Figure 1.1D). From such a configuration, we can see that the beta strands are pleated with C_α atoms successively above or below the plane of a sheet. The side chains follow this pattern as well. A beta sheet is called parallel when the strands run in the same direction or antiparallel when they do not. The example in Figure 1.1D is an antiparallel beta sheet. The beta strands are usually represented as arrows in the 3-D structure of a protein with the arrowhead pointing to the direction (N → C).

This book contains numerous structures that are represented as different models. This approach is deliberate because some models can show a particular feature much better than others. To illustrate, Figure 1.2 presents a particular structure using four different models. The structure shows the interaction between the paired domain of the activator pax-6 with DNA. It is a good example

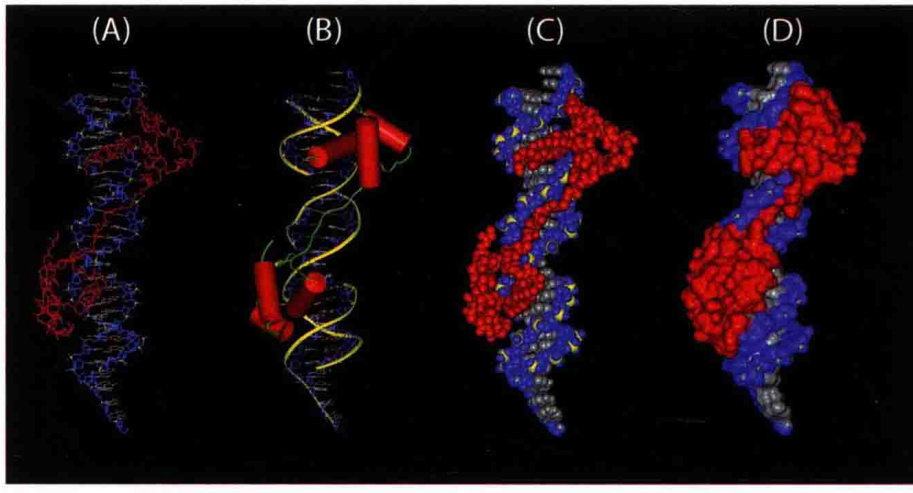

Figure 1.2. Different models of pax-6 bound to DNA. **A:** Ball-and-stick diagram. Phosphates are yellow, the sugar moiety is blue, nucleotide bases are gray, and pax-6 is red. **B:** Same as in A, but the helices of pax-6 are shown as red cylinders, and the connecting parts, as green strings. The DNA phosphates are yellow and have been traced to highlight the DNA. **C:** CPK (space-filling) model with same colors as in A. **D:** Surface representation with the same colors as in A. Images generated by E. Fuentes; Xu et al., Genes Develop. 13: 1263–75 (1999).