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PREFACE

In selecting topics for the fourth volume of the *Annual Review of Cell Biology*, our editorial committee has proceeded from the premise that, in the vast domain of contemporary life sciences, cell biology is part of a large, common, and continuous body of knowledge properly described as cellular and molecular biology. Over the last decades, this common body of knowledge has become the starting base from which biological research either has advanced in further depth in exploring patterns of organization potentially valid for all cells or has spread laterally to many biological systems differentiated by evolution itself or by scholarly traditions.

In keeping with these premises, this volume includes chapters covering new fields of research bound to affect our understanding of the functional organization of all cells, as well as research in differentiated domains dealing with prokaryotic, plant, neural, or developmental cell biology. The volume also covers cell production of macromolecules for extracellular matrices and cell interactions with such products. This active research area is beginning to provide new insights into principles and mechanisms involved in constructing supracellular levels of biological organization, in guiding developmental processes in organismic ontogeny, and in maintaining a short-range communication system on cell–extracellular matrix interactions.

Cell biology is already extensively diversified. Moreover, it is also growing simultaneously and rapidly in many different directions. It has hardly any dormant sector. Although this unusually happy situation calls for rejoicing, it also creates a few practical difficulties. For instance, it is not easy to generate a well balanced Annual Review of a large, diversified research field with so many sectors in ferment. Our editorial committee is fully aware of this problem and is trying to provide a fair and informative—yet by necessity selective—representation of work done in the entire field of cell biology. But given the inherent difficulty of the task and the diversity of the field, the committee welcomes suggestions, comments, and criticism from the readers of the *Annual Review of Cell Biology*, from the scientific community at large, and especially from the membership of the American Society of Cell Biology.

As generally recognized, cellular and molecular biology has advanced at a spectacular rate over the last decades. In the process, it has succeeded in attracting a large number of scientists originally belonging to other fields of basic or applied biological and biomedical research. We are witnessing, in fact, a movement of widespread conversion of minds and labels because the appeal generated by the concepts, technologies, and experimental strategies developed in cellular and molecular biology proves

to be stronger than the traditionally expected respect for formal discipline boundaries, which in any case no longer effectively exist. One of the immediate results of this development is than many topics that naturally belong to cell biology are covered in other Annual Review series.

There is nothing regrettable about this situation since the final result is that cell biological problems and their solutions are becoming more and more widely known. Since in the process, cellular and molecular biology is gaining rather than losing ground, there is no reason to consider any kind of defensive measures of the type applied in other countries for other important products of human endeavor. We do not need a "controlled labeling" comparable to the "appellation contrôlée" of the wines and brandies of France. For its continued success, the *Annual Review of Cell Biology* should rely on its close contact with the mainstream of research in cellular and molecular biology and on its ability to provide an integrated view—rather than disconnected glimpses—of what is going on in this amazingly fertile field.

GEORGE E. PALADE
EDITOR

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RECENT STUDIES OF DNA IN THE CRYSTAL

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CONTENTS

INTRODUCTION

In this article we review the studies that have been made of DNA in the crystal during the years 1979–1987. A review is particularly appropriate now. Seldom before has so much been learned in so short a time and yet remained unknown to all but the narrow specialist. We shall, therefore, not attempt a comprehensive review of these new results, but instead will try to present those aspects of DNA structure that promise to have a significant influence on our understanding of cell biology. In other words, this will be a review for biologists, and not for crystallographers.

The story begins with the discovery of left-handed DNA in 1979, continues with the affirmation of the sequence-dependent structure of right-

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handed DNA in 1981, then contrasts the very different structures of oligo (dG)·(dC) and oligo(dA)·(dT) in order to provide a concrete example of the role of base sequence in DNA conformation, and finishes with the outstanding example of a drug-DNA complex in which the structure of the DNA is grossly altered from the usual textbook models. Some mention is made of abnormal base pairs such as G·T, A·C, and G·A. Due to limitations of space, we cannot discuss here the structure of DNA in solution, the intrinsic curvature of DNA, or its sequence-dependent flexure around proteins. These topics, along with several useful theories of DNA structure, are presented elsewhere (Drew et al 1988).

How did this current wave of discovery begin? What was the spark that transformed the study of DNA and its physical properties from an obscure academic subject just ten years ago to an area of active enquiry today? Perhaps there will be no disagreement if we suggest that it was the discovery of left-handed DNA.

The discovery of left-handed DNA came about in the following way. Pohl & Jovin (1972) synthesized enzymatically a DNA polymer of regularly alternating G and C nucleotides, denoted as poly(dG-dC). As thorough experimentalists, they thought it would be useful to characterize the spectral properties of this polymer in solution. While doing so, they unexpectedly found that the circular dichroism spectrum of poly(dG-dC) undergoes a complete reversal in sign as the salt concentration increases from 1 to 4 M. They suspected that the DNA molecule, in going from low salt to high salt, had reversed its sense of coiling from right-handed to left-handed since such a change would account for the circular dichroism results. They were discouraged from this opinion, however, by many people. After all, DNA had been studied by fiber diffraction for over 30 years, and no left-handed helices had ever been detected with certainty.

Nevertheless, when it became possible in the mid 1970s to synthesize DNA chemically in large amounts (through the work of K. Itakura and J. van Boom) for x-ray analysis of single crystals, the salt-induced transition of poly(dG-dC) was a problem of great interest. By 1978 crystals of both d(CGCG) and d(CGCGCG) had been grown (Drew et al 1978; A. H.-J. Wang, personal communication). The x-ray patterns of these molecules in the crystal showed a helical form that had never been seen before: one with 12 base pairs per turn, a rise per base pair of 3.7 Å, and bases almost perpendicular to an imaginary helix axis. Also, the packing diameter of the helical DNA was unusually small, 18 Å, as compared to 20–23 Å for other forms of DNA.

This leads us into a description of the use of single-crystal x-ray methods and nuclear magnetic resonance spectroscopy to discover the true nature of the poly(dG-dC) structure at high and low salt.

LEFT-HANDED DNA

As mentioned above, crystals of d(CGCG) and d(CGCGCG) had been grown by 1978. There was good reason to think that the structure of the DNA in these crystals would correspond either to the low-salt or to the high-salt form of poly(dG-dC), but it was not known to which. The problem, then, was to solve the structure of DNA in the crystal and to relate its structure to the earlier solution measurements of Pohl & Jovin (1972). This was far from a trivial task. But Wang et al (1979) succeeded in preparing three heavy-atom derivatives of d(CGCGCG) and hence were able to solve its structure at 0.9 Å resolution. Aided by these results, Crawford et al (1980) and Drew et al (1980) solved two different crystalline forms of the tetramer d(CGCG) a few months later.

These structures came as a shock to everyone because all three turned out to be left-handed double helices of the kind shown in Figure 1. A left-handed helix spirals counter clockwise as it proceeds away from an observer along an axis of helical symmetry, whereas a right-handed helix spirals clockwise. The stereochemical details of left-handed DNA are too abstruse to mention here; it will suffice to say that this particular kind of left-handed helix was not predicted by anyone. Looking closely at Figure 1, which shows three d(CGCG) tetramers stacked end-on-end, one can see that the base pairs spiral in a counterclockwise sense, completing a full 360° rotation after 12 base-pair steps (bottom to top in the figure, approximately). The distance advanced along the helix axis after one turn is 12×3.7 Å $= 44$ Å. The helix is long and narrow, thereby accounting for its small packing diameter of 18 Å in crystals.

The helical parameters for right-handed DNA are very different from those just mentioned. A typical right-handed helix (such as the ones described below) completes a full 360° turn after 10.5 base-pair steps, and advances along the axis by 10.5×3.2 Å $= 34$ Å. Because the helical parameters of left-handed DNA are so different from those of right-handed DNA, the x-ray patterns obtained from crystals of d(CGCG) and d(CGCGCG) look very unusual. It is not known if other forms of left-handed DNA exist apart from the variety shown in Figure 1.

But the question remains: are the left-handed structures of d(CGCG) and d(CGCGCG) equivalent to the low-salt or to the high-salt form of poly(dG-dC)? Pohl & Jovin (1972) used very indirect methods, such as ultraviolet absorption and circular dichroism, to probe the structure of this DNA. Their studies suggested that the low-salt form might be right-handed, while the high-salt form might be left-handed, but more conclusive data were needed. The hard data were obtained from studies of poly(dG-dC) by nuclear magnetic resonance (NMR) (Patel et al 1979): NMR

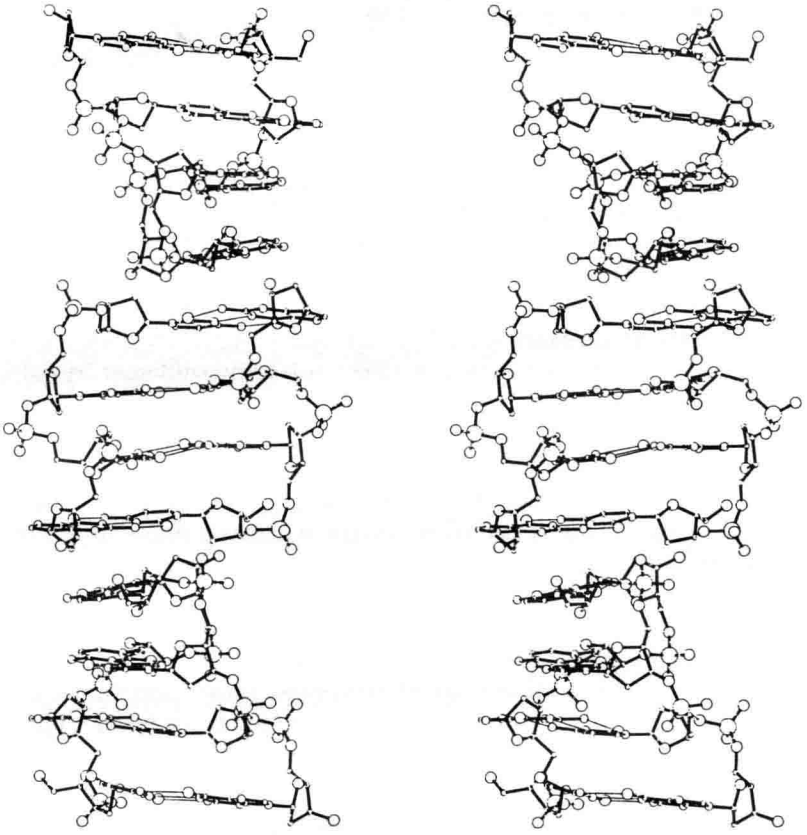

Figure 1 A stereodiagram of left-handed DNA constructed from the single-crystal x-ray coordinates of 3 d(CGCG) tetramer double helices. This kind of left-handed helix is often called Z DNA because there is a slight alternation or "zig-zag" to the arrangement of successive base pairs.

spectra show that the high-salt form of poly(dG-dC) has the symmetry of a dinucleotide, whereas the low-salt form has the symmetry of a mononucleotide. This was originally interpreted as evidence for a dinucleotide repeat at high salt in right-handed DNA; however, once the crystal structures were solved, it became clear that a left-handed helix explains the experimental results nicely. A strong dinucleotide repeat arises from the structure shown in Figure 1 as a consequence of the striking alternation in helix twist angles in going from one base-pair step to the next: The CpG step has a twist of just $-15°$ as compared to $-45°$ for the GpC step. Indeed, this left-handed helix is often called the Z form for its "zig-zag"