Instituto Juan March
de Estudios e Investigaciones

2

CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

DNA Structure and Protein Recognition

Organized by

A. Klug and J. A. Subirana

F. Azorín
D. M. Crothers
R. E. Dickerson
M. D. Frank-Kamenetskii
C. W. Hilbers
R. Kaptein
D. Moras
D. Rhodes
W. Saenger
M. Salas
P. B. Sigler
L. Kohlstaedt
J. A. Subirana
D. Suck
A. Travers
J. C. Wang

IJM
2
Wor

IJM-2-Wor

Instituto Juan March
de Estudios e Investigaciones

CENTRO DE REUNIONES INTERNACIONALES
SOBRE BIOLOGÍA

2

Workshop on

DNA Structure and Protein Recognition

Organized by

A. Klug and J. A. Subirana

F. Azorín
D. M. Crothers
R. E. Dickerson
M. D. Frank-Kamenetskii
C. W. Hilbers
R. Kaptein
D. Moras
D. Rhodes
W. Saenger
M. Salas
P. B. Sigler
L. Kohlstaedt
J. A. Subirana
D. Suck
A. Travers
J. C. Wang

Instituto Juan March (Madrid)

The lectures summarized in this publication were presented by their authors at a workshop held on the 15th through the 18th of March, 1992, at the Instituto Juan March

Depósito legal: M-15.448/1992
I.S.B.N.: 84-7919-501-0
Impresión: Ediciones Peninsular. Tomelloso, 37. 28026 Madrid.

Instituto Juan March (Madrid)

INDEX

PAGE

PROGRAMME.. 7

INTRODUCTION. A. Travers and A. Klug.................. 11

1st SESSION: DNA STRUCTURE:

R.E. DICKERSON: SEQUENCE-DEPENDENT DNA STRUCTURES AND THE RECOGNITION PROBLEM.................. 19

D. MORAS: CRYSTAL STRUCTURES OF B DNA DODECAMERS REVEAL A CROSSED STRUCTURE: IMPLICATION FOR RECOMBINATION AND MUTAGENESIS.................. 20

J.A. SUBIRANA: STRUCTURAL STUDIES WITH CG SEQUENCES... 22

Z. SHAKKED: ENVIRONMENTAL EFFECTS IN DNA CRYSTAL STRUCTURES.................. 23

2nd SESSION: DNA CURVATURE AND JUNCTIONS:

D.M. CROTHERS: DNA BENDS AND BENDABILITY.............. 27

F. AZORIN: THE INTRINSIC CURVATURE OF THE MOUSE SATELLITE DNA.................. 28

C.W. HILBERS: ANALYSIS OF DNA HAIRPIN STRUCTURES BY MEANS OF NMR MODELS FOR FOLDING IN NUCLEIC ACID MOLECULES.................. 29

W.L. PETICOLAS: CARTESIAN COORDINATES FOR STABLE B/Z AND B/Z/B JUNCTIONS IN DNA FROM RAMAN DATA AND MOLECULAR MODELING.................. 31

F. GAGO: DNA RECOGNITION BY SMALL LIGANDS. A MOLECULAR MODELLING APPROACH.................. 32

3rd SESSION: DNA POLYMORPHISM:

M.D. FRANK-KAMENETSKII: MULTISTRANDED DNA STRUCTURES.. 37

F. AZORIN: STRUCTURAL POLYMORPHISM OF D(GA·CT)$_n$ SEQUENCES: THE EFFECT OF ZINC IONS.................. 39

R.F. MACAYA: STRUCTURE AND SEQUENCE SPECIFICITY OF INTRAMOLECULAR DNA TRIPLEXES.................. 40

DISCUSSION ON DNA STRUCTURE AND POLYMORPHISM. J.A. SUBIRANA.................. 41

4th SESSION: PROTEIN-DNA RECOGNITION (I):

D. RHODES: SOLUTION STRUCTURE OF TWO DIFFERENT ZINC-BINDING PROTEIN MOTIFS USED IN DNA RECOGNITION... 47

Instituto Juan March (Madrid)

PAGE

P.B. SIGLER: DNA TARGET SELECTION BY STEROID RECEPTORS.......... 48

R. KAPTEIN: NMR STUDIES OF PROTEIN-DNA RECOGNITION: THE NUCLEAR HORMONE RECEPTORS.......... 49

D. SUCK: RECOGNITION OF DOUBLE- AND SINGLE-STRANDED SUBSTRATES BY NUCLEASES. STRUCTURAL STUDIES OF DNAse I AND P1 NUCLEASE.......... 51

5th SESSION: PROTEIN-DNA RECOGNITION (II):

M. SALAS: THE ROLE OF DNA BENDING IN THE SWITCH FROM EARLY TO LATE TRANSCRIPTION BY PHAGE Ø29 REGULATORY PROTEIN P4: ANALYSIS OF THE P4-DNA COMPLEX.......... 55

A. STASIAK: THE APICAL LOCALIZATION OF TRANSCRIBING RNA POLYMERASES ON SUPERCOILED DNA PREVENTS THEIR ROTATION AROUND THE TEMPLATE.......... 57

W. SAENGER: THREE-DIMENSIONAL STRUCTURE OF THE DNA BINDING PROTEIN FIS.......... 58

J.C. WANG: TYPE II DNA TOPOISOMERASES: MECHANISTIC STUDIES.......... 61

L. KOHLSTAEDT: COMPARISON OF THE STRUCTURES OF HIV REVERSE TRANSCRIPTASE AND THAT OF THE KLENOW FRAGMENT OF DNA POL I BOUND TO DUPLEX DNA.......... 62

A. JACOBO-MOLINA: PROGRESS ON THE STRUCTURE DETERMINATION OF A TERNARY COMPLEX OF HIV-1 REVERSE TRANSCRIPTASE, AN Fab OF A MONOCLONAL ANTIBODY, AND A dsDNA MIMIC OF THE HIV-1 PRIMER BINDING SITE.......... 63

SELECTED POSTERS ON HISTONES / CHROMATIN:

J.R. DABAN: DYNAMICS OF THE INTERACTION OF CORE HISTONES WITH SINGLE-STRANDED DNA.......... 67

J.A. SUBIRANA: α HELICAL REGIONS IN HISTONE H1, PROTAMINES AND RELATED PROTEINS.......... 68

J. BODE: SCAFFOLD-ATTACHED REGIONS (SAR ELEMENTS): INFLUENCE ON TRANSCRIPTION PREDICTED FROM STRUCTURAL AND BINDING PROPERTIES.......... 69

J.M. HERMOSO: STRUCTURE OF A MULTIMERIC NUCLEOPROTEIN COMPLEX AT THE ORIGINS OF REPLICATION OF BACTERIOPHAGE Ø29.......... 71

ROUND TABLE DISCUSSION PROTEIN-DNA RECOGNITION: R. KAPTEIN.......... 75

Instituto Juan March (Madrid)

PAGE

POSTERS (OTHER THAN THOSE PRESENTED ORALLY):

J.P. ABAD: THE G-RICH STRAND OF DODECA-SATELLITE, A TANDEM REPEATED DNA SEQUENCE FROM DROSOPHILA MELANOGASTER CENTROMERIC HETEROCHROMATIN, SHOWS UNUSUAL SECONDARY STRUCRURES "IN VITRO"........................ 79

M. COLL: X-RAY STRUCTURE ANALYSIS OF DNA AND RNA OLIGONUCLEOTIDES.. 80

G. COLMENAREJO: BINDING ISOTHERMS FOR DNA-TWO LIGANDS INTERACTION.. 81

R. FICKERT: LAC REPRESSOR FINDS LAC OPERATOR BY INTERSEGMENT TRANSFER................................. 82

P. GARRIGA: CONFORMATIONAL TRANSITION OF POLY $(amino^2dA\text{-}dT)\cdot POLY(amino^2dA\text{-}dT)$ INDUCED BY POLYAMINES. 83

J. KIM: MUTANT GCN4 PROTEINS WITH ALTERED DNA-BINDING SPECIFICITY.. 85

M.L. KOPKA: THE CRYSTAL STRUCTURE OF A COVALENT DNA ADDUCT: ANTHRAMYCIN BOUND TO CCAACGTTGG............... 86

F.J.M. MOJICA: DNA TOPOLOGY IN HALOPHILIC ARCHAEBACTERIA.. 87

J.E. PEREZ-ORTIN: FUNCTIONAL SIGNIFICANCE OF A STABLE CRUCIFORM AT THE 3'END OF THE YEAST FBP1 GENE......... 88

J. SANCHEZ: DEOXYRIBOENDONUCLEASES FROM STREPTOMYCES WHICH DO NOT BELONG TO RESTRICTION-MODIFICATION SYSTEMS... 89

M. SERRANO: BACTERIOPHAGE Ø29 dsDNA-BINDING PROTEIN: FEATURES AT THE REPLICATION ORIGINS REQUIRED TO ACTIVATE THE INITIATION OF REPLICATION AND PROTEIN MOTIF INVOLVED IN DNA BINDING......................... 90

I.Y. SKURATOVSKII: B_h-DNA: IONIC-WATER INTERACTIONS REQUIRE ADE IN NAPOLY [d(A)] : POLY [d(T)] TO BE TILTED ABOUT 12º, THE THY TILT BEING ABOUT 0.......... 91

I.Y. SKURATOVSKII: X-RAY FIBRE DIFFRACTION: X-FORM DNA = A-FORM DNA !?.............................. 92

LIST OF INVITED SPEAKERS................................... 93

LIST OF PARTICIPANTS....................................... 97

Instituto Juan March (Madrid)

PROGRAMME

DNA STRUCTURE AND PROTEIN RECOGNITION

Monday, March 16th

Introductory Remarks.
Opening: A. Travers.

1st Session. DNA Structure.

R.E. Dickerson - Sequence-Dependent DNA Structures and the Recognition Problem.

D. Moras - Crystal Structures of B DNA Dodecamers Reveal a Crossed Structure: Implication for Recombination and Mutagenesis.

J.A. Subirana - Structural Studies with CG Sequences.

Z. Shakked - Environmental Effects in DNA Crystal Structures.

Poster Session I

2nd Session. DNA Curvature and Junctions.

D.M. Crothers - DNA Bends and Bendability.

F. Azorín - The Intrinsic Curvature of the Mouse Satellite DNA.

C.W. Hilbers - Analysis of DNA Hairpin Structures by Means of NMR Models for Folding in Nucleic Acid Molecules.

W.L. Peticolas - Cartesian Coordinates for Stable B/Z and B/Z/B Junctions in DNA from Raman Data and Molecular Modeling.

F. Gago - DNA Recognition by Small Ligands. A Molecular Modelling Approach.

Tuesday, March 17th

3rd Session. DNA Polymorphism.

M.D. Frank-Kamenetskii - Multistranded DNA Structures.

Instituto Juan March (Madrid)

F. Azorín - Structural Polymorphism of $d(GA\cdot CT)_n$ Sequences: The Effect of Zinc Ions.

R.F. Macaya - Structure and Sequence Specificity of Intramolecular DNA Triplexes.

Round Table Discussion on DNA Structure and Polymorphism.

Poster Session II

4th Session. Protein-DNA Recognition (I).

D. Rhodes - Solution Structure of Two Different Zinc-Binding Protein Motifs Used in DNA Recognition.

P.B. Sigler - DNA Target Selection by Steroid Receptors.

R. Kaptein - NMR Studies of Protein-DNA Recognition: The Nuclear Hormone Receptors.

D. Suck - Recognition of Double- and Single-Stranded Substrates by Nucleases. Structural Studies of DNase I and P1 Nuclease.

Wednesday, March 18th

5th Session. Protein -DNA Recognition (II).

M. Salas - The Role of DNA Bending in the Switch from Early to Late Transcription by Phage Ø29 Regulatory Protein p4: Analysis of the p4-DNA Complex.

A. Stasiak - The Apical Localization of Transcribing RNA Polymerases on Supercoiled DNA Prevents Their Rotation around the Template.

W. Saenger - Three-Dimensional Structure of the DNA Binding Protein FIS.

J.C. Wang - Type II DNA Topoisomerases: Mechanistic Studies.

L. Kohlstaedt - Comparison of the Structures of HIV Reverse Transcriptase and that of the Klenow Fragment of DNA Pol I Bound to Duplex DNA.

Instituto Juan March (Madrid)

A. Jacobo-Molina - *Progress on the Structure Determination of a Ternary Complex of HIV-1 Reverse Transcriptase, an Fab of a Monoclonal Antibody, and a dsDNA Mimic of the HIV-1 Primer Binding Site.*

Selected Posters on Histones / Chromatin

Introduction. A. Travers

J.R. Daban - *Dynamics of the Interaction of Core Histones with Single-Stranded DNA.*

J.A. Subirana - *Helical Regions in Histone H1, Protamines and Related Proteins.*

J. Bode - *Scaffold-Attached Regions (Sar Elements): Influence on Transcription Predicted from Structural and Binding Properties.*

J.M. Hermoso - *Structure of a Multimeric Nucleoprotein Complex at the Origins of Replication of Bacteriophage O29.*

Round Table Discussion: Protein / DNA Recognition.

Instituto Juan March (Madrid)

INTRODUCTION

A. TRAVERS and A. KLUG
MCR Laboratory of Molecular Biology
Hills Road, Cambridge (UK)

Instituto Juan March (Madrid)

The conformational flexibility of both DNA and DNA-binding proteins is essential for their biological function. A major challenge is to identify the structures that are relevant to this function. A principal theme of this workshop will be the relation between the structural snapshots of a molecule or a complex in a crystal or NMR structure to the real world of the cell.

It is axiomatic that the crystal structure of a macromolecule must represent that of a particular conformer favoured by the crystallisation procedure. An important corollary is that for molecules that can adopt different conformations the biological relevance of a determined structure may be difficult to assign. DNA itself is a highly malleable polymer which can undergo structural transitions which range from global transitions such as the conversion of B- to A-DNA, to local deformations involving bending of the double-helical axis or more transient changes in base stacking or base-pair opening. A major consequence of this malleability is that the crystal packing forces can impose an overall average structure on DNA oligomers - the crystals of most dodecamers are isomorphous as, in a different form, are those of most decamers. Although such structures provide considerable insights into the structural variability of DNA their biological correlates sometimes remain elusive.

In solution the constraints of crystallisation do not apply and both the sugar-phosphate backbone and the base-pairs of DNA are structurally dynamic. Nevertheless, a particular base-step will assume an average conformation which is determined by its sequence. One consequence of this variation is intrinsic curvature the magnitude of which is a time-averaged property - the term 'static curvature' is a serious misnomer. Intrinsic curvature is conferred in particular by the occurrence of short oligo (dA).(dT) tracts in phase with the double-helical repeat. However, the precise structural basis for intrinsic curvature remains a controversial issue. In all the crystal structures of sequences containing oligo (dA).(dT) tracts the tract itself is essentially straight and the base-pairs possess a high average propeller twist. These characteristics are quite distinct from those of different A/T containing sequences in other dodecamers which form isomorphous crystals. This implies that observed structure of the (dA).(dT) tract is inherent to the sequence. This structure differs from the average conformation of flanking sequences and it is this difference that imparts curvature. An issue that remains unresolved is the relation of the structure of the tracts in the crystal to that in solution.Among the other questions that are still unanswered is the functional role of the putative water spine in the minor groove - does it even exist? We do however know that the water activity is important for the maintenance of intrinsic curvature since compounds such as dimethylsulphoxide which increase the flexibility of (dA).(dT) tracts abolish curvature. A second unresolved question is the structural basis for the loss of curvature on the binding of minor groove ligands such as distamycin. How does the interaction of such drugs differ from that of the DNA

Instituto Juan March (Madrid)

binding peptides such as SPRKSPRK, another minor groove ligand which binds selectively to the same sequences?

To what extent are the structures of DNA and protein deformed as a consequence of complex formation? To answer this question we need first to determine the solution structure of both components alone. An octamer sequence from binding site of the E. coli metJ repressor crystallises in a form with certain characteristics of A-DNA whereas in solution the structure of this same octamer, as indicated by NMR measurements, is much closer to B-form. In the crystal of the protein-DNA complex it is the latter structure that is observed, suggesting that the structure in the crystal of the DNA alone may be determined by the conditions of crystallisation. A similar congruence between the overall structure of a DNA sequence in solution and when bound by protein is apparent for the binding sites for certain zinc-finger proteins. Both when free and in the complex the DNA possesses significant A-type character.

Changes in protein structure on DNA binding can also be an important component in the formation of a DNA-protein complex. For example, the proximity of the DNA ligand can induce a coil to helix transition in the amphipathic AK regions characteristic of the C-terminal tails of histone H1 and also in the DNA-binding domain of proteins of the Bzip class. A similar stabilisation of an α-helix on DNA binding has been described for the DNA-binding domain of the glucocorticoid receptor. Another functionally significant transition is the encirclement of the DNA substrate by the EcoRV restriction endonuclease. Such ligand dependent transitions allow the establishment of structures which could not be attained by a simple docking procedure. By contrast the structure of DNase I is essentially the same when crystallised as the free protein or as a complex with its substrate. Again the structure of individual zinc fingers of the C-C....H-H (TFIIIA) class in solution as determined by NMR is, on average, very similar to that when bound to DNA in the Zif268-DNA cocrystal. However, an NMR study of a two-finger peptide clearly indicates that the structurally independent zinc finger domains are connected by a flexible linker. Therefore the structure adopted on interacting with a DNA target site is determined by the geometry of interaction of individual finger domains with the double helix - the linkers play a purely passive role.

There are many examples of DNA deformation induced by protein binding where the structural properties of the DNA sequence itself influences the interaction of the protein. Notable cases include the endonuclease DNase I which bends the double-helical axis by ~25° away from the protein, the phage 434 cI and Cro repressors which narrow the minor groove between the two helix-turn-helix contact points and the Trp repressor which bends the DNA at three sites, all of which contain thermally labile TA steps. Even more extreme distortions are induced by proteins whose primary function is to bend DNA. Substantial bending is induced by the E. coli factor for inversion

Instituto Juan March (Madrid)

stimulation (Fis) and catabolite activator protein (CAP), which deflect the double-helical axis through an estimated 65° and 90° respectively over 25-30 base-pairs. In the cocrystal of CAP with its DNA binding site this latter bend is largely a consequence of the complete disruption of base-stacking at two 40° 'kinks' half a double-helical turn on each side of the dyad. By contrast in the nucleosome core particle where the DNA is deflected on average by 47° for each double helical turn, the bend, as judged by a lack of chemical reactivity, is more evenly distributed.

The problem of the selection of a particular conformer by crystallisation is potentially even more acute when the molecule under study can function as part of a multicomponent complex. Again CAP is a pertinent example. It is known that the CAP dimer can assume more than one structure. By itself the protein crystallises so that the two monomers possess different conformations, whereas, by contrast, in the cocrystal the conformations of the two monomers are essentially identical. What then is the conformation of the complex in solution? The bases exposed at the kinks would be expected to be accessible to chemical reagents yet in solution are unreactive with singlet oxygen.However, when CAP activates transcription initiation at the lac promoter the reactivity of these bases to this reagent changes profoundly. This suggests that the local conformation of the DNA bound by CAP alters significantly during the structural transitions required for the establishment of a competent initiation complex and that structure of the DNA in the cocrystal may be that of one of several possible relevant states.

What is the function of DNA bends of the magnitude induced by the histone octamer or by CAP? A major role must be to order the path of the DNA double helix in large protein-DNA complexes and so facilitate the formation of appropriate contacts. In the nucleosome the bending permits efficient packaging but for CAP the bend must perform a different function. A surprising feature of ternary complexes of CAP and RNA polymerase bound at a promoter site is that the protein interactions extend upstream beyond the established binding sites for either protein alone. The inference from this and related observations is that the DNA bend induced by CAP allows the polymerase to contact a second region of DNA thus delimiting a loop. Put another way, optimal recognition of a promoter site by the enzyme requires a specified DNA configuration.

This brings the argument back full circle to DNA. If a protein can specify a defined DNA configuration the inherent flexibility of the DNA molecule alone might achieve the same purpose. In principle DNA bending in a relatively small region of anisotropically flexible DNA could determine the global configuration of a DNA domain. In the energetically favoured interwound form of negatively supercoiled DNA the strain is not distributed uniformly. Such molecules must contain at least two loops, the apices, connecting the interwindings. Within these loops the DNA is tightly bent and consequently bendable DNA would be expected to be preferentially located at these positions. Such an effect would limit the available configurations imposing an entropic

Instituto Juan March (Madrid)

penalty. However this cost could be offset by the generation of a uniquely configured protein binding site. Are there examples of naturally occurring sequences which might occupy such loops? One candidate is the anisotropically flexible sequence which constitutes the extended promoter DNA of the tyrT gene, one of the most highly expressed genes in E.coli. Not only is this DNA bendable but it contains phased binding sites for the Fis protein. In other words, it is designed to be bent. In vivo the superhelical density of bacterial DNA varies substantially and is at a maximum on entry into the exponential phase of growth - at precisely the time when the intracellular concentration of Fis is highest. Thus a possible function for Fis in this context could be to stabilise the promoter DNA in a apical loop and locally minimise the dynamic fluctuations inevitable in a highly supercoiled DNA molecule.

The dynamic nature of DNA molecules and of many of their interactions with proteins require conformational flexibility. The structures of biological macromolecules in the crystal and in solution are both impressive and highly informative We hope that this meeting will extrapolate from these inevitably static images to the true biological context of the observed structures. Finally, on behalf of both ourselves and of Juan Subirana we would like to thank the Juan March Foundation, and Andrés González in particular, for making it all possible.

Andrew Travers and Aaron Klug
Cambridge, March 1992

Instituto Juan March (Madrid)

1st Session:
DNA STRUCTURE

Instituto Juan March (Madrid)

Sequence-Dependent DNA Structures and the Recognition Problem

Richard E. Dickerson
Molecular Biology Institute
University of California
Los Angeles CA 90024

X-ray crystal structure analyses of DNA oligomers and of their complexes with groove-binding antitumor drug molecules to date have all involved 12-base-pair double helical dodecamers, of sequence: C-G-C-G-A-A-T-T-C-G-C-G or its variants. The drug binding site has been the central AT-rich region, with an unusually narrow minor groove that leads to a snug fit of an organic ring such as pyrrole, or of a planar amide. All dodecamers have a common crystal packing, in which the AT-rich center of the minor groove is not in close contact with neighboring molecules of the crystal, thus allowing the accommodation of a drug molecule.

Attention has shifted recently to 10-base-pair decamers, that stack atop one another to simulate infinite columns of helices running through the crystal, with a significant improvement in resolution of the analysis. These analyses have shown that an AT base pair region need not necessarily have a narrow minor groove: the groove is narrow in the dodecamer C-G-C-A-T-A-T-A-T-G-C-G, but wide at the center of C-G-A-T-A-T-A-T-C-G. Clearly DNA is a malleable object, subject to deformation by local forces, whether these arise from crystal neighbors, bound drugs, or proteins to which the DNA is bound. The sequence-related limits to this local malleability are under investigation.

The tight monoclinic and orthorhombic packing modes of decamers that produce such excellent resolution in crystal structure analyses of DNA alone may be too tight to accommodate bound drug molecules. Repéated crystallization attempts have failed to produce crystals of DNA-drug complexes with these decamers. The best matrix for further DNA-drug binding studies may be the old dodecamers with varying choices of central eight base pairs: C-G-x-x-x-x-x-x-x-x-C-G, sacrificing resolution for drug accessibility.

The DNA double helix is neither a rigid rod nor a formless mass of spaghetti. Different regions of base sequence influence the static structure or deformation of the helix. But they also influence the dynamic deformability of that same helix, leading, for example, to differential susceptibility to bending or twist. Both components are important in recognition of particular DNA base sequences by drugs and proteins.

Instituto Juan March (Madrid)

Crystal Structures of B DNA Dodecamers Reveal a Crossed Structure : Implication for Recombination and Mutagenesis

Dino Moras, Youri Timsit and Eric Vilbois

Laboratoire de Cristallographie Biologique,
Institut de Biologie Moléculaire et Cellulaire
Centre National de la Recherche Scientifique
15, rue Descartes, 67084 Strasbourg-Cedex, France

The crystal structures of d(ACCGGCGCCACA) (tet) and d(ACCGCCGG-CGCC) (ras) have revealed a new kind of packing of B-DNA helices and have allowed the observation of important structural alterations. Both sequences correspond to natural DNA fragments containing the NarI site GGCGCC, a hot spot for N2-acetyl-amino-fluorene mutagenesis : tet is the most mutagenic site of PBR322 (1) and ras corresponds to the codons 10-13 of the wild type Ha-ras protooncogene. In this fragment, the NarI site is adjacent to the 5'end of codon 12 where single point mutations activate the ras protooncogene in many tumors (2). The structures were refined to 15.3 % and 16.1 % at 2.6 A resolution, respectively.

Crystal packing analysis has revealed an unexpected intermolecular interaction between the B-DNA duplexes : the helices are locked together by the reciprocal fit of the sugar-phosphate backbone in the major groove and form a crossed structure. Specific H-bonds are formed between the phosphate anionic oxygen atoms of the backbone and the amino group of the alternate cytosine bases C3/C21 and C6/C18 (3). This self fitting property of B-DNA could be a model for DNA - DNA interaction in various biological processes such as the DNA condensation and the recombination (4). The crossed structure formed by two interacting helices is also a good starting point to study Holliday intermediates. Magnesium plays a crucial role in the stabilization of the crystals and greatly influences their diffracting power. Its localization in the crystal structures allows to understand how its participation to the intermolecular solvent bridges between the duplexes can contribute to the stabilization of the crystal

Instituto Juan March (Madrid)

packing. This constitutes a structural basis to analyse the involvment of divalent cations in the conformation and dynamic of Hollidays intermediates.

The analysis of the -10 degrees structure of the tet dodecamer reveals that the cooling stabilizes a new conformational state with a long range sequence dependent one - step - shift in the major groove base pairing (5). This alteration propagates along the helical axis over more than half a turn. The comparison with the isomorphous ras structure shows that the neighbouring sequences affect the extent of local packing induced alteration. This structural property which allows more flexibility in the complementary base pairing rules should be considered in the analysis of the various processes involving the decoding of the genetic information and could be a molecular model for understanding the mechanisms of frame - shift or dislocation - substitution mutagenesis.

(1) Koffel-Schwartz, N., et al. J. Mol. Biol. 177, 33-51 (1984)

(2) Reddy, E.P, Reynolds, R.K., Santos, E. & Barbacid, M., Nature 300, 149- 152 (1982).

(3) Timsit, Y., Westhof, E., Fuchs, R. & Moras, D. Nature 341, 459-462 (1989)

(4) Timsit, Y. & Moras, D. J. Mol. Biol. 221, 919-940 (1991)

(5) Timsit, Y., Vilbois, E. & Moras, D. Nature 354, 167-170 (1991)

Instituto Juan March (Madrid)

STRUCTURAL STUDIES WITH CG SEQUENCES

N. Verdaguer, L. Urpí, I. Fita and J.A. Subirana
Departament d'Enginyeria Química, Universitat Politècnica de Catalunya, Diagonal 647, 08028 Barcelona

The conformation of DNA in fibers is determined by the sequence and the environment (counterions, humidity). In oligonucleotide crystals additional factors are involved, such as the length and the terminal bases, which obscure the relationship that may exist between the parameters observed in the crystal structure and the solution conformation of DNA

We have recently determined the structure of the dodecamer CCCCCGCGGGGG (Verdaguer et al, J. Mol.Biol. **221**, 623, 1991). It shows some peculiarities which may be of interest to understand the conformational features mentioned above:

1st. It shows that dodecamers, that usually crystallize in the B form, can also crystallize in the A form.
2nd. The dodecamer is already in the A form in the solution from which it has been crystallized (Kypr et al, unpublished observations)
3rd. It crystallizes with the terminal base pairs tucked into the shallow groove of neighbouring molecules as in all A form crystals studied up to now.
4th. The two CpG steps have different conformational parameters which also differ from those measured in other sequences. It appears that this step has a large conformational variability.

We have also studied the hexamer sequence CCGCGG which is identical to the central sequence of the dodecamer. We find that this hexamer may crystallize in the B and Z forms and possibly also in the C form. Thus this sequence is very versatile, since it may adopt all the known DNA conformations depending on the crystallization conditions and on the sequences around it.

These results will be compared with previous studies with CG sequences. In summary, it can be concluded that:

1st.) End and length effects are important in the crystalline structure of oligonucleotides, besides the well known influence of sequence and counterions.
2nd.) In order to understand sequence effects we should not only take into account the favoured conformations found in crystals, but also their intrinsic variability.

Instituto Juan March (Madrid)

ENVIRONMENTAL EFFECTS IN DNA CRYSTAL STRUCTURES

Zippora Shakked

Department of Structural Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

The conformational diversity of the DNA double helix is largely dependent on its environment. The data on DNA structures determined by X-ray crystallography demonstrate that while certain sequences are structurally rigid, other sequences are inherently flexible and easily deformed by environmental factors such as crystal packing, solvent molecules and interacting proteins. Environmental effects in DNA crystal structures have been studied by our group in the following three systems:

(1) Polymorphic structures: The same A-DNA duplex d(GGGCGCCC), has been crystallized in three different crystal forms. The comparative analysis of the three structures has demonstrated major conformational changes from one crystal environment to the next depending on hydration, temperature and intermolecular interactions.

(2) Isomorphic structures: Different oligomers crystallizing in the same crystal form are subjected to similar environmental effects. This is the case of two newly determined B-DNA dodecamers d(CGCGATATCGCG) and d(CGCAIATCTGCG) which are isomorphous to several other B-DNA crystal structures. A comparison between the two structures shows that global

Instituto Juan March (Madrid)

bending of these duplexes is induced by crystal packing as for all the other members of this series. However, the specific bending pathway is dictated by the base sequence. Other features such as minor-groove dimensions and base-stacking interactions of the AT-rich regions are intrinsic to the base sequence with only slight modulations as a result of environmental effects.

(3) DNA operators: Two fragments of DNA operators were crystallized: the *trp* operator d(TGTACTAGTTAACTAGTAC) and the *met* operator d(CTAGACGTCTAG). Crystal structures of the complexes of DNA with the corresponding repressor proteins were already determined by X-ray crystallography and thus a comparison between the naked and complexed molecules can be used to monitor structural changes in the DNA which are induced by the interacting proteins. The common feature of the two operator crystals is the unusual large number of unique copies of DNA duplexes in the unit cell indicating that several conformations are trapped during crystallization probably as a result of the deformable nature of these particular sequences. The crystal structure of the *met* fragment has already been solved showing three unique duplexes which display a common overall structure of the B-type-DNA with significant variations in local structure between the different molecules.

Instituto Juan March (Madrid)

2nd Session:
DNA CURVATURE AND JUNCTIONS

Instituto Juan March (Madrid)

DNA Bends and Bendability

Donald M. Crothers, Department of Chemistry
Yale University, New Haven, CT 06511 U.S.A.

The DNA helix axis is straight only under idealized conditions. Bending of the molecule can result from flexing under thermal fluctuations, from intrinsic regularities in the sequence, and from stress induced by binding proteins and other ligands. DNA flexibility can be characterized by the persistence length, which is about 150 base pairs for DNA of average sequence. It is not presently known how much this parameter varies within the set of naturally occurring DNA sequences.

Intrinsic bending or curvature of DNA results when certain base sequences or structural motifs are repeated in phase with the DNA helical repeat (≈10.5 bp/turn). In this arrangement the small curvature contributions of the repeating elements add coherently because they are all on the same side of the helix. Among natural sequences, the main contributor to intrinsic DNA curvature is the "A-tract", which consists of homopolymeric dA•dT base pairs, usually about half a helical turn long. The resulting bent DNA molecules can be characterized by comparing their electrophoretic mobility with that of normal DNA. Using this technique, and varying the phasing between different bends to assess their relative direction, we have concluded that A tracts cause DNA to bend in a direction which is toward the minor groove in a coordinate frame located near the center of an A_{5-6} tract. This result is consistent with simple geometric considerations when the A tract bend is compared with bends produced by *E.coli* CAP protein, by an extra base on one strand, and by the G-G intrastrand croslink resulting from reaction of *cis*-diamminedichloroplatinum (II) with DNA. In addition, we have measured the extent of DNA curvature by determining the relative rate of cyclizing molecules containing phased A tracts, with the conclusion that each tract bends the helix axis by $18 \pm 2^{\circ}$. The structural basis for bending includes narrowing of the minor groove.

The bendability of DNA when interacting with proteins depends on base sequence. The rules we have found for this process in the interaction of *E. coli* CAP protein are generally similar to those seen for DNA bending in nucleosomal complexes: A•T rich sequences are preferred when the minor groove is compressed, whereas G•C rich regions are better if the major groove is compressed. These effects are also correlated with binding strength, albeit imperfectly: weaker binding generally accompanies weaker bending. In additional studies with transcription complexes we have found that the bend induced in DNA by CAP protein is maintained and probably even enhanced in the ternary open complex containing CAP and RNA polymerase.

Intrinsic DNA bends can sometimes replace the function of protein-induced bends. We have studied this effect in the context of the *E. coli lac* promoter. The rate of forming open promoter complexes is enhanced as much as ten fold in promoter constructs having the CAP binding site replaced by phased A tracts, provided that the intrinsic DNA bend is in the same direction as the natural bend produced by CAP. These results suggest a role in gene activation for the bend itself, in addition to possible effects due to contacts between CAP and polymerase.

Two proteins can act synergistically in binding to DNA if they both prefer that DNA be bent in the same direction. We have explored the potential magnitude of this effect by measuring the binding affinity of CAP protein for its binding site in small circles, compared to the affinity for linear fragments. Effects of more than two orders of magnitude are observed.

Instituto Juan March (Madrid)

THE INTRINSIC CURVATURE OF THE MOUSE SATELLITE DNA.

P.Carrera, M.A.Martinez-Balbás, N.Ferrer, J.Portugal[1] and F.Azorín.
Department of Molecular and Cellular Biology. CID-CSIC.
[1] Department of Biochemistry. University of Barcelona.

In this paper we analyze the intrinsic curvature of the mouse satellite DNA. The 243 bp. long repeating unit contains nineteen groups of three or more consecutive adenines which are only poorly phased with respect to the helical repeat. This DNA fragment shows properties which are characteristic of curved DNA. Its migration in polyacrylamide gels is slower than predicted from its sequence showing an R_L factor of 1.41 .It also shows a sinusoidal pattern of cleavage by the hydroxyl radical; the waves of reactivity being phased with respect to the A-tracts. Some interesting observations arise from a detailed analysis of the hydroxyl radical cleavage patterns: a) the maxima of hydroxyl radical cleavage are more periodically spaced along the DNA sequence than the A-tracts themselves. As a consequence, the position of each maximum with respect to the A-tract is variable; b) the sequence 5´ TGGAATAT$^{G}/_{A}$A 3´ shows a sinusoidal pattern of hydroxyl radical cleavage. This sequence shows a retarded migration in polyacrylamide gels indicating that it is actually intrinsically curved. The intrinsic curvature of the 5´ TGGAATATGA 3´ sequence is associated to the precise primary structure of the central A+T-rich part. Permutation of the position of the AA dinucleotide significantly affects the curvature of the corresponding DNA fragments which, at some positions, is completely abolished. These results are discussed in view of the current models for DNA curvature.

Instituto Juan March (Madrid)

ANALYSIS OF DNA HAIRPIN STRUCTURES BY MEANS OF NMR MODELS FOR FOLDING IN NUCLEIC ACID MOLECULES.

C.W. Hilbers, M.J.J. Blommers, M.M.W. Mooren and S.S. Wijmenga.
Nijmegen SON Research Centre, Faculty of Science, University of Nijmegen, The Netherlands.

J.H. van Boom and G.A. van der Marel, Department of Organic Chemistry, State University of Leiden, Leiden, The Netherlands.

NMR and optical studies have demonstrated that DNA oligomers can form stable monomeric hairpin structures, sometimes even when these oligomers are self complementary. The stability of the hairpins varies, however, depending on the base pair composition and sequence in the stem region and depending on the nucleotide sequence of the loop portion of the molecule. A large number of NMR studies have been performed to unravel how the structure of the loop region is affected by its nucleotide sequence and how in turn this influences the hairpin stability.

In our initial studies we developed a simple 'geometric' model to describe loop structure formation. It was assumed that base stacking, as it occurred in the stem region, was continued into the loop region . As a result it was expected that in DNA hairpins with a B-type stem this base stacking in the loop would be continued from the 5'-end into the 3'-direction while in RNA hairpins it would be the other way around. To close the loop a sharp turn in the sugar phosphate backbone was expected to occur in DNA hairpins after the third nucleotide and in RNA hairpins after the fifth or sixth nucleotide. Examples which fulfilled this folding pattern were found in tRNA and in a DNA hairpin. The model also allows for base pair formation of the terminal bases of the loop sequence and this was observed indeed but depending on the loop sequence.
The construction of detailed structural models for the loop region is very often hampered by the paucity of the NMR constraints. The quality of the data we obtained for the partly self complementary hexadecanucleotide d(ATCCTA-TTTA-TAGGAT) permitted, however, a more in a depth study of the loop structure. It was found that the terminal T and A residues of the loop sequence form a Hoogsteen base pair with A in a syn-conformation. The second thymidine is turned into the minor groove but the phosphate backbone still follows a course as expected from the 'geometric model' mentioned above. This includes the occurrence of the sharp turn after the third thymidine leading to a reversal of the direction of the sugar phosphate backbone. The detailed conformation of this sharp turn can be rationalized on the basis of structural data available for smaller systems.

These studies were extended to hairpins formed by the sequences d(ATCCTA-GTTA-TAGGAT) and d(CGCGCG-TTT-CGCGCG).
It is wellknown that hairpins with the loop sequence -GNRA- (where N can be any nucleotide and R is either A or G) frequently occur in 16S and 23S ribosomal RNA. Moreover, the isolated hairpins show an enhanced stability.

Instituto Juan March (Madrid)

This was also found for DNA hairpins with G and A in these loop positions. It was surmized that this might result from the possible formation of a G-A base pair in the loop region. This was indeed borne out of NMR experiments on RNA as well as DNA hairpins. Our NMR experiments show that in the DNA hairpin formed by d(ATCCTA-GTTA-TAGGAT) a G-A base pair is formed. Just as in the hairpins with the -TTTA- loop, the A in the -GTTA- loop is turned into the syn-domain and this allows formation of a G(anti)- A(syn) base pair in which the distance between the C1' sugar carbons is 10.7 Å. This base pair can be easily accomodated in a B-type double helix. Again, as in the hairpin with the -TTTA- loop, one of the T's in the -GTTA- loop is turned into the minor groove.

A similar picture evolved for the hairpin formed by the sequence d(CGCGCG-TTT-CGCGCG). In contrast to most of the hairpins studied so far this hairpin has an uneven number of nucleotides in the loop. Moreover, this sequence is interesting because at low pH it forms a triple helix with a -TTT- loop in a different setting.

The structure of the loops formed by d(ATCCTA-GTTA-TAGGAT) and d(CGCGCG-TTT-CGCGCG) were studied extensively by distance geometry and NOE back calculations.

As mentioned in all of these examples a thymine residue is turned into the minor groove. The stabilities available for hairpins with T's in the loop region are increased with respect to related hairpins in which they are replaced by other bases. The possible anchoring of thymidine in the minor groove may provide an explanation for this observation.

Instituto Juan March (Madrid)

Cartesian Coordinates for Stable B/Z and B/Z/B Junctions in DNA from Raman Data and Molecular Modeling by Warner L. Peticolas, Zhongi Dai, G. A. Thomas, and Manuel Dauchez. Department of Chemistry, University of Oregon, Eugene, Oregon 97403 USA

A method has been developed to obtain the Cartesian coordinates of oligonucleotides containing a B/Z or B/Z/B junction in solution and in micro-crystals. In this method the oligonucleotide is sequenced in such a say to keep the B and Z portions of the chain separate. Oligonucleotides of the form 5'-d[$T_N(CG)_M$] and their complex conjugate were synthesized as well as self complimentary oligomers of the form 5'-d[$T_N(CG)_MA_N$]. Raman marker bands for the AT base pairs showed that they were always in the B form while in 6M NaCl solution, a certain fraction of the CG base pairs were found to be in the B-form and the rest in the Z-form. To obtain the Cartesian coordinates of these oligomers, the module BIOPOLYMER of the SYBYL molecular modeling package was used on an IRIS 4D computer. Energy minimization calculations were carried out using the AMBER 3.0A all atom force field that is implemented in the SYBYL program (version 5.4). Calculations were performed for an isolated oligomer duplex with the effect of solvent taken into account by taking the dielectric as a function of distance with a maximum distance cutoff of 10 A. Counterions were not included explicitly but their effect was modeled by partially reducing the charge on each phosphate group. The minimum energy configuration for these B/Z and B/Z/B junctions did not permit any base pairs to be in the junction because of the separation of the Watson-Crick hydrogen bonds. This model for the B/Z junction was refined to be in excellent agreement with the Raman intensity data which gave the number of CG base pairs in the B and in the Z form. To test our model further, C_8-H to C_8-D hydrogen/deuterium experiments were carried out on the oligomer duplex,

5'-d(TTTTTTCGCGCGCGCGCGCGAAAAAA)$_2$

It was found that in the B form the HD exchange can be expressed as the sum of a slower rate due to the AT base pairs and a higher rate due to the CG base pairs. In 6M salt, two rates were observed for the CG base pairs--a slow rate due to the CG base pairs in the B form and a fast rate due to the CG base pairs in the Z form. No evidence is found for CG base pairs in a "junction". The enthalpy for the formation of the junction was measured from melting experiments and is approximately the positive enthalpy for the loss of a single base pair stacking interaction per junction. Our model for the B/Z/B junction indicates that the B form starts at each end in the AT base pairs and is propagated 4 CG base pairs into the center from each side. This leaves 8 CG base pairs in the center in the Z form. The junction on each side of the Z form is very abrupt. After the last CG base pair in the B form the next CG base pair is flipped out to start the Z form. This abrupt change in the conformation from B to Z may account for some of the unusual reaction properties of these junctions. The Cartesian coordinates of these junctions are obtainable from the authors.

Instituto Juan March (Madrid)

DNA recognition by small ligands. A molecular modelling approach.

Federico Gago and José Gallego - Universidad de Alcalá de Henares (Madrid)

Sequence-selective DNA binding is a property shared by some proteins that interact with DNA and by some comparatively small ligands such as certain antitumour drugs and short peptides (1). In some instances it has been shown that both types of molecules compete for the same sites on the double helix (2), which has led to speculation about the mode of action of these drugs (1). This most probably arises from the drug interfering with the recognition and function of DNA binding proteins and is best documented in the case of topoisomerase II-targeted anthracyclines (3).

In addition to several experimental approaches, molecular modelling techniques and accurate theoretical calculations can be of value in order to improve our understanding of the forces involved in the recognition process. In this respect, we focused first on a group of non-intercalating DNA-binding drugs that show a preferential binding to runs of A,T bases over G,C pairs in double-stranded DNA. The calculated binding enthalpies to poly[d(AT)]·poly[d(AT)] and poly[d(GC)]·poly[d(GC)] satisfactorily correlated with the selectivity ratio provided by experiment (4). Moreover, the models suggested that differences in minor groove widths in both types of complexes could be relevant to the specificity of the interaction. The thermodynamic cycle perturbation approach was then used to calculate the difference in free energy of binding of netropsin to poly[d(IC)]·poly[d(IC)] and poly[d(GC)]·poly[d(GC)]. The theoretical value of 4.3 kcal mol^{-1} agreed well with the experimentally determined value of 4.0 kcal mol^{-1} and the width of the minor groove was again the most notable structural feature distinguishing the two complexes (5).

In another sudy, the structure of the Hoechst 33258 molecule, another typical minor groove ligand, was used to model putative bioreductive DNA binding ligands (6). These molecules would present the interesting property of being active binders in their reduced form, which will predominate in oxygen-deficient tumour cells, but not in their oxidized form.

Among the topics we are currently addressing in our laboratory are new chromophores interacting with DNA through intercalation and molecular dynamics calculations on triostin A and echinomycin-DNA complexes.

Instituto Juan March (Madrid)

References

1. Churchill, M. E. A. & Travers, A. A. (1991) *Trends Biochem. Sci.*, 16, 92-97.

2. Suzuki, M. (1989) *EMBO J.*, 8, 797-804.

3. Liu, L. F. (1989) *Annu. Rev. Biochem.*, 58, 351-375.

4. Gago, F., Reynolds, C. A. & Richards, W. G. (1989) *Molec. Pharmacol.*, 35, 232-241.

5. Gago, F. & Richards, W. G. (1990) *Molec. Pharmacol.*, 37, 341-346.

6. Haworth, I. S., Burt, C., Gago, F., Reynolds, C. A. & Richards, W. G. (1991) *Anti-Cancer Drug Design*, 6, 59-70.

Instituto Juan March (Madrid)

3rd Session:
DNA POLYMORPHISM

Instituto Juan March (Madrid)

Multistranded DNA Structures

Maxim D. Frank-Kamenetskii, Boris P. Belotserkovskii, Vlad A. Malkov, Alexei G. Veselkov, Oleg N. Voloshin and Valery N. Soyfer*

Institute of Molecular Genetics, Russian Academy of Sciences, Moscow 123182, Russia

*Laboratory of Molecular Genetics, George Mason University, Fairfax, VA 22030, USA

A variety of DNA structures, which include more than two strands, has been studied. The best studied examples of such multistranded structures are inter- and intramolecular pyrimidine-purine-pyrimidine (PyPuPy) triplexes and H-DNA, which includes such triplex as a major element. They are formed under acidic conditions and, in case of H-DNA, under superhelical stress. More recently, pyrimidine-purine-purine (PyPuPu) triplexes, inter- and intramolecular and within the PyPuPu analog of H-DNA (H*-DNA), has been studied. These triplexes are pH-independent and are usually stabilized by bivalent cations.

Using photofootprinting assay (detecting both, [6-4] photoproducts and cyclobutane photodimers) and dimethyl sulfate footprinting we have studied the formation of intermolecular PyPuPu triplex for different sequences in the presence of a variety of bivalent cations: Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}. The data obtained exhibit a number of intriguing peculiarities. We found that without triplex formation, the $d(C)_{18}$ $d(G)_{18}$ duplex itself shows remarkable decrease of photoreactivity with respect to formation of cyclobutane dimers in the presence of Ba^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}. In the absence of bivalent cations or in the presence of Mg^{2+} or Ca^{2+} the tract shows normal photoreactivity. We conclude that the $d(C)_n$ $d(G)_n$ duplex adopts in the presence of Ba^{2+}, Cd^{2+}, Co^{2+},

Instituto Juan March (Madrid)

Mn^{2+}, Ni^{2+} or Zn^{2+} a non-B-conformation.

The PyPuPu triplex is formed in the presence of most of the tested bivalent cation but effect of some cations depends on the sequence. Specifically, Zn^{2+} stabilizes both, $d(C)_n$ $d(G)_n$ $d(G)_n$ and $d(CT)_n$ $d(GA)_n$ $d(GA)_n$ triplexes, whereas Mg^{2+} stabilizes the former but not the latter. In the presence of Zn^{2+}, we have detected the formation of PyPuPu triplex for an irregular purine oligonucleotide and corresponding duplex with mirror-repeated sequence. These data made it possible to conclude that within the PyPuPu triplex the two purine strands have anti-parallel orientation with respect to one another.

The both classes of triplexes characterized to date, PyPuPy and PyPuPu, are symmetrical in the sense that the same bases are present in both pyrimidine strands (in the case of PyPuPy triplex) or in both purine strand (in the case of PyPuPu triplex). Only solitary deviations from such symmetry (mismatches) were described, which significantly destabilized triplexes. We have found that, under acidic conditions, the stable asymmetrical triplex between oligo (GA) and $d(C)_n$ $d(G)_n$ duplex is formed, consisting of CGG and CGA^+ base-triads.

In the course of our studies of superhelically-induced unusual DNA structure we have observed the formation of a protonated unusual structure by human telomeric sequence, $(T_2AG_3)_n$ $(C_3TA_2)_n$. We have probed the structure by a variety of chemical agents as well as with S1 nuclease. We have concluded that under superhelical stress and acid pH the $(T_2AG_3)_n$ $(C_3TA_2)_n$ sequence adopts an unusual structure in which two multistranded DNA elements coexists. Specifically, for n = 12 three T_2AG_3 blocks enter an unorthodox triplex, half of base-triads in which are non-canonical. Four T_2AG_3 blocks in the single-stranded region of this pseudo-H-DNA, which we called "eclectic structure", form quadruplex.

Instituto Juan March (Madrid)

STRUCTURAL POLYMORPHISM OF d(GA·CT)$_n$ SEQUENCES: THE EFFECT OF ZINC IONS.

F.Azorín, R.Beltrán, J.M.Casasnovas, J.Bernúes, A.Martínez-Balbás, A.Huertas and M.Ortiz
Department of Molecular Biology.
Centro de Investigación y Desarrollo, CSIC.
Jorge Girona Salgado 18-26, 08034 Barcelona, Spain.

Homopurine-homopyrimidine d(GA·CT)$_n$ sequences are structurally polymorphic. It has been shown that upon protonation they undergo transition to a triple-stranded conformation. Recently, we reported a zinc-induced structural transition of a d(GA.CT)$_n$ sequence[1,2]. This novel structure, called *H-DNA, is induced at neutral pH by moderate concentrations of zinc. Stabilisation of this altered conformation appears to be specific of nucleotide sequence and metal-ion. In this paper we will discuss the following aspects: (1) the determination of the conditions (degree of supercoiling, metal-ion concentration, ionic strength, pH, etc.) for the stabilisation of *H-DNA; (2) the determination of the conformation adopted by d(GA·CT)$_n$ sequences in the presence of zinc. The modification patterns obtained with several chemical reagents (DMS, DEPC, OsO_4, MnO_4K, CAA, BAA) indicate that, at relatively low Zn/P ratios, the sequence adopts a Pur-Pur-Pyr triplex. Upon increasing the Zn/P ratio, the pyrimidine strand gets completely unpaired, while the purine strand remains associated by intrastrand pairing (GA-loop); (3) characterisation of the types of intra- and interstrand pairing of d(GA)$_n$ and d(CT)$_n$ sequences in the absence and presence of zinc, and (4) the possible role of this type of repeated sequences on DNA recombination[3].

1.- Bernúes et al., (1989) EMBO J. 8, 2087.
2.- Bernúes et al., (1990) Nucleic Acids Res. 18, 4067.
3.- Bernúes et al., (1991) Gene, in press.

Instituto Juan March (Madrid)

Structure and Sequence Specificity of Intramolecular DNA Triplexes

Román F. Macaya, Dara E. Gilbert, Peter Schultze, Edmond Wang, Shiva Malek, and Juli Feigon.

Chemistry and Biochemistry Dept., University of California, Los Angeles, 405 Hilgard Ave., Los Angeles, California 90024

We have used 2D NMR to investigate the structural characteristics and sequence specificity of intramolecular DNA triplexes. These triplexes are formed from 31 and 32 base oligonucleotides which fold into intramolecular triplexes containing eight base triplets. The third strand in triplexes composed of T·AT and C^+·GC triplets binds in the major groove parallel to the purine strand via Hoogsteen base pairs. Analysis of the coupling constants from the sugar protons in such a triplex indicates that, contrary to the widely used Arnott model, most of the sugars in these triplexes assume primarily the S-type rather than the N-type conformation. Therefore binding of the third strand in the major groove does not require all the sugars in the triplex to adopt 3'-*endo* sugar puckers.

The structure and relative stability of X·GC mismatches (where X is any nucleotide) in intr_molecular tripiexes were determined. Although the triplexes containing A^+·GC, G·GC, and T·GC triplets are less stable than the corresponding parent molecule containing a C^+·GC triplet, all mismatched bases form specific hydrogen bonds in the major groove.

Instituto Juan March (Madrid)

DISCUSSION ON DNA STRUCTURE AND POLYMORPHISM

J. A. Subirana
Departamento de Ingeniería Química UPC
E.T.S.I. Industriales
Barcelona (Spain)

Instituto Juan March (Madrid)

Discussion on DNA structure and polymorphism
(Summary by J.A. Subirana)

"DNA does not think like we do", with these words R. Dickerson started the general discussion. Indeed, as a consequence of the presentations at the meeting and current work elsewhere, it was apparent that DNA has a changing structure, with some sequences more rigid than other, but the rules that determine its shape under any conditions are not clear. The conformation of each nucleotide is determined by six torsion angles which can not vary in an arbitrary fashion, but no clear simplification of the six dimensional space has thus far emerged. The situation is particularly complex when DNA interacts with proteins which may easily disturb its conformation, not only producing bends and kinks, but also other unexpected states. The work of Suck et al, for example, has shown intermediate conformations between the A and B forms which had never been detected in isolated DNA molecules.

The conformation of DNA is usually characterized by geometrical parameters (twist, inclination, slide, etc.) which depend on the torsion angles, but define in a more direct way the variations of the double helical structure. These parameters do not vary independently, there are subsets of them which are directly related, but we do not yet know all the possible interplays among them. We would like to know not only the average values, but also the resistance to change of all these parameters as a function of sequence.

It was considered that **further studies of identical sequences in different environments** may help to understand the variability of DNA structure since many examples are already available which show that the same DNA sequence may adopt different conformations in different environments (change in crystal form, counterions, presence of proteins, etc.).

In particular some discussion centered around the rigidity of adenine tracts or the factors which determine groove width in DNA, features which are strongly related to water hydration, bending, drug binding, etc. It became clear that groove width may vary as a consequence of different changes in the parameters of DNA structure (twist, roll, propeller, etc.), so that the apparent shape of DNA depends on subtle changes of its conformational

Instituto Juan March (Madrid)

parameters. Different sequences may be sensed by proteins as equivalent, as it is the case for the recognition sites of FIS protein. On the other hand no clear definition of the "rigidity" of adenine tracts could be achieved, although it is apparent that such tracks have a rather uniform, rigid conformation, whereas alternating AT regions may vary much more locally.

DNA in a dilute salt solution may not be the most interesting case to study, but it is the reference state. We would like to know how it responds upon interaction with its neighbour molecules, in packing (viruses, chromosomes) or interacting with proteins. After all, packing forces in a crystal may be of interest since they allow an interaction of DNA with its neighbour molecules. Water structure around DNA may be part of the protein recognition process, even involving water molecules which are not located in the recognition site.

In summary, the same sequence may show different conformations, whereas different sequences may show similar features!!

The discussion finished with some comments on multistranded structures, where it is obvious that much more structural work is required both with X-ray diffraction and NMR. In particular it is not clear what is the degree of order found in single stranded loops, since different techniques yield apparently contradictory results.

Instituto Juan March (Madrid)

4th Session:
PROTEIN-DNA RECOGNITION
(I)

Instituto Juan March (Madrid)

SOLUTION STRUCTURE OF TWO DIFFERENT ZINC-BINDING PROTEIN MOTIFS USED IN DNA RECOGNITION.

Daniela Rhodes, John Schwabe, David Neuhaus.

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

Amongst the different designs for sequence specific DNA recognition that have emerged so far, the "zinc-finger" appears to be the one used most frequently. This protein motif was first recognized in the transcription factor TFIIIA, but has now been found within the sequence of at least 200 proteins, and occurs in combinations of 2 to 37 fingers repeated in tandem. A zinc ion forms the basis of the tertiary folding by being tetrahedrally coordinated to one pair of cysteines and one pair of histidines (C_2-H_2 type). A second class of zinc-binding motifs, containing only cysteines as zinc-ligands (C_2-C_2 type), are found in pairs and constitute the DNA-binding domain of the steroid and thyroid hormone receptor proteins.

We have used 2D-NMR spectroscopy to elucidated the 3D-structure of both classes of zinc-finger motifs. The first is a peptide which contains two C_2-H_2 type fingers from the DNA-binding domain of the yeast transcription factor SWI5. The second is the DNA-binding domain of the human oestrogen receptor. Despite similarities in the primary sequence of these zinc-binding motifs, their secondary and tertiary structures are distinctly different. More strikingly, whereas the zinc-fingers of SWI5 form independently folded mini domains, the zinc-binding motifs of the oestrogen receptor fold together to form a single structural domain.

These structures together with biochemical data give insight into how these small protein domains direct the highly specific DNA recognition necessary for the control of gene expression.

1) Neuhaus, D., Nakaseko, Y., Nagai, K. and Klug, A., F.E.B.S.Lett, 262: 179 (1990)
2) Schwabe, J.W.S., Neuhaus, D. and Rhodes, D., Nature, 348: 458 (1990)
3) Schwabe, J.W.S.and Rhodes, D., T.I.B.S., 16: 291 (1991)

Instituto Juan March (Madrid)

DNA TARGET SELECTION BY STEROID RECEPTORS

WeiXin Xu, Ben F. Luisi, and Paul B. Sigler

Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT 06510, USA.

Steroids and other lipophylic hormones or ligands (like thyroxin vitamin D, retinoic acid, etc.) convey metabolic and developmental signals to the cell where they modulate transcription from hormone-responsive promoters. The mechanism is mediated through a large homologous family of hormone-specific receptors which are transcription factors. These factors are 'activated' by the hormone and targeted to the appropriate regulatory element near the responsive genes. The receptor's DNA-binding domain contains two Zn-nucleated modules which fold together to form a unified globular domain. This domain, which is sufficient for accurate targeting, behaves as a monomer in solution. In the more straightforward cases, these DNA targets, called hormone response elements, are composed of six-base pair 'half-sites' arranged either as inverted or direct repeats. The half-sites contain a set and arrangement of bases common to all response elements and usually two-base pairs that are specific for the particular steroid receptor. Thus the sequence of the half-site is partly responsible for targeting a particular receptor to its cognate promoter. However, the orientation and spacing of the half-sites also contributes to the target's identity. For example, the estrogen and thyroid response elements have the same half-site sequence arranged as inverted repeats; however, they are distinguished by the presence of a three-base pair spacer between the half-site in the ERE and the absence of any spacer in the TRE.

The crystal structures of the DNA-binding domain of the glucocorticoid receptor in a complex with an idealized response element (and also one with an extra base pair in the spacer) have been solved and refined. These structures show the mechanism by which both the half-site sequence and the spacer is recognized. The recognition of the spacer requires that the normally monomeric DNA-binding domains form tight dimers which firmly place the recognition surfaces of each subunit in correct register with the identity elements of the half-sites. This is accomplished through the allosteric effect of the DNA itself. Phosphate interactions (which are largely sequence *in*dependent) with the side chains of the second Zn-finger induce secondary and tertiary structures which we know from NMR analyses are not present in the unbound protein. These allosterically induced structural elements form a strong dimer interface locking the two DNA-binding domains into an orientation whose recognition surfaces complement the identity elements of appropriately spaced half-sites.

Further studies with DNA-binding domains produced by mutagenesis will be discussed as well as progress towards understanding 'cross talk' between the receptors and more proximal protein components of the transcriptional regulatory apparatus.

Instituto Juan March (Madrid)

NMR Studies of Protein-DNA Recognition: The Nuclear Hormone Receptors

R. Kaptein

Bijvoet Center for Biomolecular Research
University of Utrecht, Padualaan 8
3584 CH Utrecht, The Netherlands

Nuclear hormone receptors constitute a family of eukaryotic transcription factors that mediate the effect of small molecule ligands such as the steroid and thyroid hormones, vitamin D_3 and retinoic acid. These proteins have a modular architecture with separate domains for ligand binding, DNA binding and transactivation. The DNA-binding domains (DBD's) are highly homologous among the family and consist always of two zinc-fingers of the type where Zn^{2+} is coordinated by four cysteine residues. We have determined the structure of the DBD's of two members of the family, the glucocorticoid receptor (GR) (1,2) and the retinoic acid receptor (RAR) by multidimensional NMR.

For the glucocorticoid receptor EXAFS studies (3) had previously shown that two Zn^{2+} ions are tetrahedrally coordinated by cysteinate anions. However, since the DBD's of all members of the family contain 9 conserved cysteine residues, it was not known which 8 of these are involved in metal binding. By exchanging Zn for ^{113}Cd (one of the magnetic isotopes of cadmium) and using ^{113}Cd-^{1}H heteronuclear NMR experiments, we have now established the correct coordination scheme (4), which is as shown in Figure 1.

The three-dimensional structure of a 93 amino acid residue GR DBD fragment was determined from distance constraints from ca. 600 nuclear Overhauser effects (NOE's) and from the Zn coordination in the zinc fingers (1). The most prominent structural feature of the protein domain is that there are two α-helices in the regions following the fingers that cross perpendicularly to form a hydrophobic protein core. One of these helices acts as a recognition helix by binding in the major groove of DNA.

A model proposed for the complex of GR-DBD with its glucocorticoid response element (GRE) (1) was recently supported by a crystallographic study of this protein-DNA complex (5). The major difference with the solution structure was located in the D-box (cf. Fig. 1) and a few other residues of the second zinc finger that are involved in protein-protein interaction in the dimeric complex. This region which appears to be rather flexible in solution is much more structured in the complex. An NMR study of the GR-DBD complexed with a half-site GRE is in progress (6).

Instituto Juan March (Madrid)

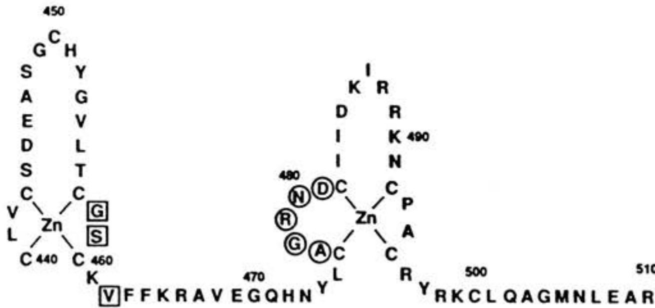


Figure 1. Amino acid sequence of the structured region of the glucocorticoid receptor DNA-binding domain (numbering is from rat GR). Boxed residues belong to the "recognition helix" and are involved in DNA recognition. Circled residues constitute the D-box and are involved in dimerisation (protein-protein interaction), which occurs upon DNA binding.

All trans-retinoic acid (RA) plays a prominent role in vertebrate development and cell differentiation. The DNA-binding domain of the retinoic acid receptor (RAR) shows a 40% sequence homology with that of GR. It is therefore no surprise that three-dimensional structures of the DBD's of the two receptors are rather similar. The structure of the human RARβ DBD determined from ca. 900 NOE distance constraints differs only in a few minor aspects, such as the length of the α-helices and a better structured C-terminal region, from that of GR. However, DNA binding by the RAR is different from that of GR. While glucocorticoid response elements are palindromic and have a strict spacer requirement of three base pairs between the half-sites, for RAR both direct and inverted repeat response elements occur and spacings between half-sites vary. The consequences of the structure of the RAR-DBD for DNA binding specificity have been examined by model building techniques.

REFERENCES

1 T. Härd, E. Kellenbach, R. Boelens, B.A. Maler, K. Dahlman, L.P. Freedman, J. Carlstedt-Duke, K.R. Yamamoto, J.-Å. Gustafsson, and R. Kaptein, *Science* (1990) 249, 157-160.

2. T. Härd, E. Kellenbach, R. Boelens, R. Kaptein, K. Dahlman, J. Carlstedt-Duke, L.P. Freedman, B.A. Maler, E.I. Hyde, J.-Å. Gustafsson, and K.R. Yamamoto, *Biochemistry* (1990) 29, 9015-9023.

3. L.P. Freedman, B.F. Luisi, Z.R. Korszun, R. Basawappa, P.B. Sigler and K.R. Yamamoto, *Nature* (1988) 334, 543-546.

4. E. Kellenbach, B.A. Maler, K.R. Yamamoto, R. Boelens and R. Kaptein, *FEBS lett.* (1991) 291, 367-370.

5. B.F. Luisi, W.X. Xu, Z. Otwinowski, L.P. Freedman, K.R. Yamamoto, and P.B. Sigler, *Nature* (1991) 253, 497-505.

6. M.L. Remerowski, E. Kellenbach, R. Boelens, G.A. van der Marel, J.H. Boom, B.A. Maler, K.R. Yamamoto and R. Kaptein, *Biochemistry* (1991) 30, 11620-11624.

Instituto Juan March (Madrid)

Recognition of double- and single-stranded substrates by nucleases. Structural studies of DNase I and P1 nuclease.

D. Suck, European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, Germany.

Recognition of double-stranded nucleic acids by proteins involves interactions with the functional groups of the bases as well as contacts to the sugar-phosphate backbone. The specific contacts to the bases allow a direct "readout" of the sequence, while the mainly electrostatic interactions with the backbone enable an indirect "readout" of the base sequence via the recognition of sequence-dependent variations of the helix geometry. A growing number of high resolution structural studies has provided insight into the mechanism of recognition of double-stranded substrates but relatively little is known on the structural level about the interactions of proteins with single-stranded DNA or RNA. A common feature of these interactions appears to be the stacking of exposed aromatic protein side chains with the nucleobases.

Bovine pancreatic DNase I shows a strong preference for double-stranded substrates and cleaves DNA with markedly varying cutting rates depending on the base sequence. The crystal structures of two DNase I complexes with octanucleotide duplexes (d(GCGATCGC)$_2$ (Octa I) and d(GGTATACC)$_2$ (Octa V)) have been solved and refined at high resolution (Lahm & Suck, 1991; Lahm et al., 1991). In both complexes the binding of DNase I induces local as well as global distortions in the DNA substrate. In particular, the tight interactions of a tyrosine (Y76) and an arginine (R41) in the minor groove combined with multiple contacts to both sugar-phosphate backbones leads to a widening of the minor groove and a bending towards the major groove (Figure 1).

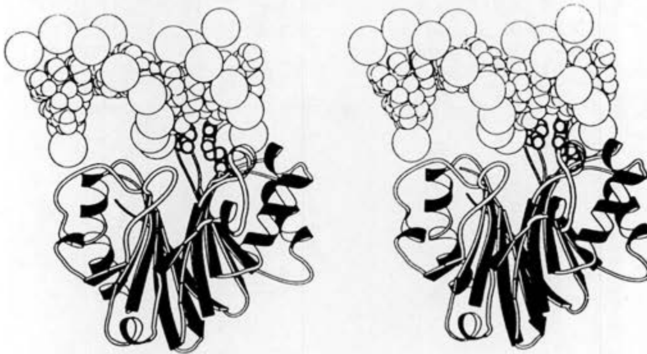


Figure 1: DNase I - Octa I complex. Y76 and R41 (shown in space-filling mode) form tight interactions in the minor groove of the DNA.

While the general architecture, the type of interactions and the overall geometry of the DNA is the same in both complexes, there are significant differences in local DNA structure which have to be attributed to the different sequences. The most noticeable difference is the spatial orientation of the base pairs which in Octa V is close to that generally observed in A-DNA and may explain the relative resistance of the d(GGTATACC)$_2$ duplex. The X-ray results lead to the conclusion that minor groove width and depth and the bendability of DNA are the most important parameters determining the cutting rates.

Other than in the DNase I - Octa I complex is the oligonucleotide in the DNase I - Octa V complex not cleaved. The disposition of residues around the scissile phosphate suggests, in

Instituto Juan March (Madrid)

agreement with site-directed mutagenesis experiments, that two histidines, H134 and H252, are involved in catalysis.

In contrast to DNase I, P1 nuclease from *Penicillium citrinum* shows strong preference for single-stranded substrates and cleaves DNA and RNA in an essentially unspecific reaction. The 3D-structure of the zinc-dependent enzyme (270 amino acids; approximately 20% carbohydrate) has been determined at 2.8Å from a tetragonal crystal form (Volbeda et al., 1991) and more recently at 2.2Å from an orthorhombic crystal form. The protein contains 14 α-helices accounting for 56% of the structure and has a fold very similar to that of phospholipase C (PLC) from *Bacillus cereus* despite very limited sequence homology. A cluster of 3 zinc ions is found at the bottom of the active site cleft, inaccessible to any double-stranded DNA or RNA, readily explaining the preference of the enzyme for single strands (Figure 2). The arrangements of the zinc ions and - with one exception - also their ligands are identical in P1 and PLC, strongly suggesting an evolutionary relationship. Two nucleotide binding sites approximately 20Å apart have been identified by soaking the crystals with thiophosphorylated substrate analogues. At both sites an adenine base is bound in a hydrophobic pocket, formed by a phenylalanine (F61) and a valine (V132) at the active site and by two tyrosines (Y144, Y155) at the other site. Based on the observed binding of the nucleotide a mechanism is proposed involving the nucleophilic attack by a zinc-activated water molecule at the phosphorous.

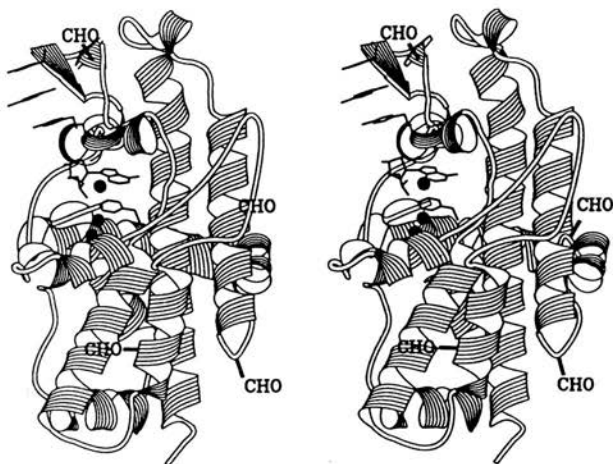


Figure 2: Stereo ribbon plot of P1 nuclease. Black dots represent the 3 zinc ions, "CHO"-labels indicate the four Asn-linked carbohydrate side chains. A 5'-AMP is shown bound at the active site stacked onto F61. At a second site an adenine ring is stacked between two tyrosines.

Lahm, A. & Suck, D. (1991) J. Mol. Biol. **221**, 645-667.

Lahm, A., Weston, S.A. & Suck, D. (1991) Nucl. Acids Molec. Biol. **5**, 171-186.

Volbeda, A., Lahm, A., Sakiyama, F. & Suck, D. (1991) EMBO J. **10**, 1607-1618.

Instituto Juan March (Madrid)

5th Session:
PROTEIN-DNA RECOGNITION
(II)

Instituto Juan March (Madrid)

The role of DNA bending in the switch from early to late transcription by phage Ø29 regulatory protein p4: analysis of the p4-DNA complex.

Margarita Salas, Fernando Rojo, Beatriz Nuez and Manuel Serrano
Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049-Madrid (Spain).

Transcription of *B. subtilis* phage Ø29 late genes occurs from the PA3 promoter, located close to the divergently transcribed main early promoter, PA2b. The viral early protein p4 is a transcriptional regulator which activates transcription from the late promoter while simultaneously repressing the early PA2b promoter (1,2). Protein p4 binds to a region of the genome located between nt -56 to -102 relative to PA3 transcription start site (-16 to -62 relative to PA2b), recognizing two 8 bp inverted sequences (3). This region of the promoter has a sequence-directed curvature that increases considerably when protein p4 binds to it (1,4). The induction of this curvature appears to be relevant both for the activation of PA3 and for the repression of PA2b.

Regarding PA3 activation, curvature could both provide a DNA conformation that stabilizes RNA polymerase binding to the promoter and, at the same time, orient protein p4 towards the RNA polymerase so that adequate contacts can be made. Protein p4 mutants that produce only a partial bending of the promoter fail to activate transcription, supporting the importance of the induced DNA curvature (4). On the other hand, several data indicate that protein p4 and RNA polymerase bound at PA3 contact each other. Both proteins bind to the promoter cooperatively, stabilizing each other in their binding sites (3). When protein p4 binding site was moved away from that of the RNA polymerase at PA3 by 1, 2 or 3 complete helical turns activation was still possible if the intervening DNA was bent, but not if this DNA was not bent or if separated by half a turn (5). This suggests that protein p4 needs to be stereospecifically oriented relative to the RNA polymerase for a correct interaction, and that activation at a distance requires the formation of a DNA loop.

Activation of PA3 is paralelled by the repression of the main early promoter, PA2b, which partially overlaps with protein p4 binding site and is transcribed in a direction opposite to that of PA3. Indeed, the binding of protein p4 displaces the RNA polymerase from the early PA2b promoter while favouring its binding to the late PA3 pomoter. Although steric hindrance is possibly involved in protein p4 repression of PA2b, the p4-induced DNA bending also seems to participate in the repression process. When the DNA curvature of protein p4 binding site was progressively increased by site-directed mutagenesis in the same direction as protein p4 does, without affecting critical positions of the early promoter, it became apparent that DNA bending by itself can inhibit transcription from PA2b (2). This bending affected both the rate of formation of transcriptionally active open complexes and the affinity of the RNA polymerase for PA2b promoter. Protein p4-induced DNA bending could therefore participate in PA2b

Instituto Juan March (Madrid)

repression by producing a DNA structure that prevents the RNA polymerase from recognizing the promoter. This mechanism would complement the steric hindrance increasing the repression efficiency, since competition between the regulator and the RNA polymerase for their binding sites would be considerably diminished.

References- (1) Barthelemy & Salas (1989) *J. Mol. Biol.* **208**, 225-232; (2) Rojo & Salas (1991) *EMBO J.* **10**, 3429-3438.; (3) Nuez et al. (1991) *Nucl. Acids Res.* 19, 1337-2342; (4) Rojo et al. (1990) *J. Mol. Biol.* **211**, 713-725; (5) Serrano et al. (1991) *J. Mol. Biol.* **219**, 403-414.

Instituto Juan March (Madrid)

The apical localization of transcribing RNA polymerases on supercoiled DNA prevents their rotation around the template.

A. Stasiak, B. ten Heggeler Bordier, M. Adrian, W. Wahli and J. Dubochet
Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne-Dorigny, Switzerland.

The interaction of Eschcrichia coli RNA polymerase with supercoiled DNA was visualized by cryo-electron microscopy of vitrified samples and by classical electron microscopy methods. wc observed that when E. coli RNA polymerase binds to a promoter on supercoiled DNA, this promoter becomes located at an apical loop of the interwound DNA molecule. During transcription RNA polymerase shifts the apical loop along the DNA, always remaining at the top of the moving loop. Since an apical loop in supercoiled DNA always points towards the outside of the circular DNA molecule, and since the position of the enzyme is defined with respect to the plane of the apical loop, RNA polymerase does not circle around DNA but forces the DNA to rotate around its axis. This interplay between RNA polymerase and the supercoiled DNA template precludes circling of the RNA polymerase around the DNA and prevents thc growing RNA transcripts from becoming entangled with the template DNA.

Instituto Juan March (Madrid)

Three-Dimensional Structure of the DNA Binding Protein FIS

D. Kostrewa, J. Granzin, C. Sandmann, U. Lücken D. Stock, W. Saenger

Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, 1000 Berlin 33, FRG

The factor for inversion stimulation, FIS, is involved in several cellular processes, including site-specific recombination and transcriptional activation. In the reactions catalysed by the DNA invertases Gin, Hin and Cin, FIS stimulates recombination by binding to an enhancer sequence. Within the enhancer, two FIS dimers (each 2 x 98 amino acids) bind to two 15-base-pair consensus sequences and induce bending of DNA. Current models propose that the enhancer FIS complex organizes a specific synapse, either through direct interactions with Gin, or by modelling the substrate into a configuration suitable for recombination.

Using X-ray analysis at 2.0 Å resolution, we have shown that FIS is composed of four α-helices A, B, C, D. In the dimer formed by FIS, helices A, A', B, B' are arranged in a four-helix-bundle to which are attached helices C, D and C', D' as helix-turn-helix motifs. The first twenty or so (depending on refinement) N-terminal amino acids are not seen in the electron density which suggests that they are either dynamically or statically disordered in the crystal lattice (1).

As shown by footprint experiments, FIS binds to two adjacent major groups along the DNA double helix. The separation between the floors of these grooves is 29 Å, but the separation between helices D and D' that presumably bind to these major grooves is only 29 Å. This implies that either FIS has to change its structure when binding DNA or, conversely, DNA has to bend so that the separation between adjacent major grooves becomes shortened. The latter is in agreement with gel shift retardation experiments which indicate that FIS bends DNA upon binding at an angle of about 90°. Using the structure of DNA as observed in the nucleosome core, we were able to match a smoothly bent DNA to the helix-turn-helix motifs of the FIS dimer (Fig. 1). This is probably an oversimplification as biochemical

Instituto Juan March (Madrid)

Figure 1. Computer-modeled complex between the FIS dimer and smoothly bent DNA. Helices D, D' are inserted in adjacent major grooves, N-termini are to the right of the picture. The structure is twofold-symmetrical with the rotation axis horizontal.

Instituto Juan March (Madrid)

data have shown that certain exposed parts of DNA become hypersensitive to nuclease and chemical attack when binding to FIS. These studies suggest that FIS does not bend smoothly around DNA but that it bends with kinks that are located symmetrically to follow the overall two-fold symmetry of the FIS-DNA complex.

To investigate the bending of DNA by FIS in more detail, we have initiated cryo-electronmicroscopic studies. A piece of DNA was synthesized, cloned and isolated in larger quantities with FIS binding sites located every 63 base-pairs (6 turns of double stranded DNA); the total length of the DNA molecule contains four of these binding sites. The binding of FIS to this DNA produced kinks of about 90°, as expected.

For one mutant of FIS, Thr87Ala, it was shown that the bending angle of DNA is not 90° but significantly smaller (2). We have crystallized this mutant to see whether the mutation modifies the separation of the helix-turn-helix motifs, thereby changing the bending angle of DNA. It is clear from the X-ray analysis that the structure of the mutant is practically identical with that of wildtype FIS. This suggests that it is the interaction between Thr87 and the DNA that influences the bending angle.

References:

1. D. Kostrewa, J. Granzin, C. Koch, H.-W. Choe, S. Raghunathan, W. Wolf, J. Labahn, R. Kahmann, W. Sanger (1991) Nature 349, 178-180.

2. R. Osuna, S.E. Finkel, R.C. Johnson (1991) EMBO J. 10, 1593-1603.

3. C. Koch, O. Ninnemann, H. Fuss, R. Kahmann (1992) Nucl. Acids Res. in press.

Instituto Juan March (Madrid)

Type II DNA Topoisomerases: Mechanistic Studies. James C. Wang, J.E. Lindsley, P.R. Caron, R.A. Wasserman, J. Bedard, and J.M. Berger, Department of Biochemistry and Molecular Biology, Cambridge, Massachusetts, 02138, USA.

Type II DNA topoisomerases are enzymes that catalyze transient breakage of double-stranded DNA for the passage of one double-stranded DNA segment through another. They are also DNA-dependent ATPases, and the binding and hydrolysis of ATP are coupled to the manipulation of DNA by these enzymes. Several aspects of these enzymes will be summarized and discussed: (1) the common features of type II DNA topoisomerases encoded by genes of a diverse collection of organisms ranging from bacteriophage to human; (2) how might these enzymes interact with DNA; (3) the use of epitopically end-labeled enzymes in the study of allosteric changes induced by ATP-binding; (4) the use of yeast in the study of anticancer-drugs targeting eukaryotic type II DNA topoisomerase.

Instituto Juan March (Madrid)

COMPARISON OF THE STRUCTURES OF HIV REVERSE TRANSCRIPTASE AND THAT OF THE KLENOW FRAGMENT OF DNA POL I BOUND TO DUPLEX DNA

Thomas A. Steitz, Lori Kohlstaedt, Jimin Wang, Jonathan Friedman, Phoebe Rice and Lorena Beese, Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University, New Haven, CT 06511 USA

Both the reverse transcriptase (RT) from HIV and the Klenow fragment (KF) of DNA polymerase I from E. coli are DNA polymerases, the former accepting either RNA or DNA as template and primed initially by $tRNA^{Lys}$, and the latter accepting only DNA as template. We have determined the crystal structures of KF with bound duplex DNA and dNTP substrates as well as that of HIV RT. RT is a heterodimer of a 66 kD subunit containing a polymerase and an RNase H domain and a 51 kD subunit containing only the polymerase domain. A 3.5 Å resolution map of the heterodimer crystallized in space group C2 with a=122.6, b=70.2, c=106.4 Å, β=105.6° shows that the two subunits are not related by a simple rotation axis and the two polymerase domains have strikingly different conformations in spite of having identical sequences. The structures of the polymerase domains of these two enzymes are clearly related though not identical. The site for duplex DNA bound to KF is in an unexpected location that is, however, consistent with the relative positions of the RNase H and polymerase domains of RT, assuming that these two enzymes function similarly.

Instituto Juan March (Madrid)

PROGRESS ON THE STRUCTURE DETERMINATION OF A TERNARY COMPLEX OF HIV-1 REVERSE TRANSCRIPTASE, AN Fab OF A MONOCLONAL ANTIBODY, AND A dsDNA MIMIC OF THE HIV-1 PRIMER BINDING SITE.

Alfredo Jacobo-Molina[1], Raymond G. Nanni[1], Arthur D. Clark, Jr.[1], Roger L. Williams[1], Xiaode Lu[1], Jianping Ding[1], Anqiang Zhang[1], Stephen H. Hughes[2], and Edward Arnold[1], 1-Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854, and Department of Chemistry, Rutgers University, 2-ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201.

Reverse transcriptase (RT) has been identified as an essential player in retroviral replication and is, to date, the target of the only approved treatments against human immunodeficiency virus type-1 (HIV-1) infection. It is expected that the improvement of existing inhibitors against RT, or the design of new ones, will be greatly facilitated when the 3-D structure of RT is determined. Many laboratories, including our own, have found it difficult to obtain high resolution crystals of HIV-1 RT. In order to overcome this problem, we assessed the relative affinities of a panel of nine mAbs : HIV-1 RT and selected a subset of Fabs for cocrystallization experiments. We obtained two different crystal forms of HIV-1 RT p66/p51 heterodimer and Fab28, corresponding to the mAb with the highest affinity for the enzyme [Jacobo-Molina *et al., PNAS* 88, 10895-10899 (1991)]. RT:Fab28 form II crystals diffracted to 4 Å resolution using the F1 beamline at the Cornell High Energy Synchrotron Source (CHESS) and showed diffuse scattering. When the form II crystals were grown in the presence of synthetic dsDNAs that mimic the HIV-1 primer binding site (PBS), crystals of the ternary complex were obtained. The RT:Fab:dsDNA crystals are isomorphous with the RT:Fab28 form II crystals, diffract X-rays to 3.1 Å resolution, and belong to the trigonal space group $P3_112$ with cell dimensions of a = b = 168.7 Å, and c = 220.3 Å. In addition, the diffuse scattering of the RT:Fab28:dsDNA crystals was reduced, and the crystals were less sensitive to radiation damage. Surprisingly, soaking of the form II crystals with the dsDNAs produced crystals with the same properties as those cocrystallized in DNA, and the improvement on diffraction resolution was dependent on the length of the dsDNA used. Scaling of diffraction intensities implies the presence of pseudosymmetry within the crystallographic asymmetric unit. We have recently calculated MIR . Å resolution electron density maps based on heavy atom derivative phasing followed by solvent flattening, independent for both the non-DNA and DNA containing crystals. The dsDNA used was a 19:18-mer with a 5' overhang that simulates the primer-template substrate at the HIV-1 PBS. A substantial portion of the DNA appears to be ordered and is located on a cleft on the surface of the protein seen in the non-DNA map. In addition, the difference Fourier of the DNA minus the non-DNA data shows a continuous density with dimensions of up to 20 Å wide and 60 Å long, that resembles a double helical structure. Features corresponding to the major and minor grooves of the DNA can be identified. The difference map also showed density that could correspond to protein movements upon binding of the DNA, and/or portions of the protein that are not ordered in the non-DNA crystals and that may be participating in substrate binding. The results suggest that the dsDNA is potentially placed in the binding site of RT in a biologically relevant fashion, and therefore this work will correspond to the first report of a structure of a polymerase:primer-template complex in which the nucleic acid is ordered. We are currently analyzing on native and derivative datasets of the RT:Fab28:dsDNA crystals collected to 3.5 Å resolution.

Instituto Juan March (Madrid)

SELECTED POSTERS ON HISTONES / CHROMATIN

Instituto Juan March (Madrid)

DYNAMICS OF THE INTERACTION OF CORE HISTONES WITH SINGLE-STRANDED DNA

Xavier Fernàndez-Busquets and Joan-Ramon Daban
Departament de Bioquímica i Biologia Molecular, Fac. Ciències, Universitat Autònoma de Barcelona, 08193-Bellaterra(Barcelona)

We have previously studied various altered nucleosome core particle structures probably involved in the natural mechanism of nucleosome self-assembly [A.M. Aragay, P. Diaz & J.R. Daban (1988) *J. Mol. Biol. 204,* 141-154; A.M. Aragay, X. Fernàndez-Busquets & J.R. Daban (1991) *Biochemistry*, 30, 5022-5032]. All these studies have been carried out with double-stranded DNA (dsDNA). However, taking into account that during replication and transcription of chromatin the normal structure of the nucleosome must be strongly perturbed to allow the transient formation of single-stranded DNA (ssDNA), in the present work we have investigated the interaction of core histones with ssDNA. We have used ssDNA from bacteriophage M13 and the purified (+) and (-) strands (158 nucleotides) of a cloned DNA fragment from nucleosomal origin. Nondenaturing gel electrophoresis shows that histones have a high affinity for ssDNA at physiological salt concentrations. Core histones are able to protect ssDNA from digestion by micrococcal nuclease. Histone-ssDNA complexes show a high tendency to aggregate in 0.2 M NaCl, but at lower ionic strength nucleosome-like structures can be seen in the electron microscope. The apparent binding constant corresponding to the association of histones with M13 dsDNA is equivalent to that found for M13 ssDNA. Competitive association experiments confirm these observations. However, we have found that the purified (+) strand of the cloned nucleosome DNA used in this study has a higher affinity for histones than the purified complementary (-) strand. This preferential binding suggests that the association of histones with one strand could occur spontaneously during replication or transcription. Furthermore, our results indicate that the length of DNA (number of nucleotides in ssDNA or base pairs in dsDNA) associated with a histone octamer is the same for both ssDNA and dsDNA. This may facilitate the dissociation of the two strands without alteration of the linear order of histones along DNA detected by other authors [V.V. Shick *et al.* (1980) *J. Mol. Biol. 139,* 491-517] with crosslinking methods. Finally, we have observed using different experimental approaches that core histones do not preclude the reassociation of the two complementary strands of DNA. In the presence of histones there is no change in the rates of reassociation of the denatured M13 strands and of the separated (+) and (-) strands of the cloned nucleosome DNA.

Instituto Juan March (Madrid)

α HELICAL REGIONS IN HISTONE H1, PROTAMINES AND RELATED PROTEINS

N. Verdaguer, M. Perelló and J.A. Subirana

Dept. Enginyeria Química. E.T.S.Enginyers Industrials U.P.C.
Diagonal 647, E-08028 Barcelona, Spain.

The spermatozoa of higher vertebrates contain protamines with a typical composition (about 60% Arg) quite different from histones. Among lower vertebrates and invertebrates, species are found with many different protein compositions which cover the whole range between histones and arginine rich protamines. An analysis of some of these proteins indicates that protamines may share some common structural features with the C-terminal region of histone H1.

The C-terminal region of Histone H1 has been shown to have a rather uniform charge distribution (1) and a tendency to form α-helical regions (1,2) in helicogenic solvents ($NaClO_4$ or trifluoroethanol). In this communication we show that a 16 amino acid long peptide with a sequence found in sea urchin sperm H1 is practically 100% α-helical. This peptide is amphipatic and may associate through its alanine rich side, while the other side interacts with DNA. This type of interaction might occur in internucleosomal DNA and play a role in the stabilization of chromatin fibers.

The ϕ_0 protein is found in sea cucumber spermatozoa, together with a standard complement of histones. It is 77 amino acids long, with a composition similar to the C-terminal region of histone H1, but with many arginines present instead of lysines. It represents 4% of the total histone complement. We have found that this protein is also fully helical in helicogenic solvents and may represent a special intermediate case between the C-terminal region of histone H1 and protamines. Chromatin fibers from this material have a rather uniform size (3). This protein may play the role of the C-terminal region of histone H1 present in conventional chromatin and stabilize its higher order structure.

Protamines from different sources show a lower but significant amount of helical content, which may remain stable upon interaction with DNA.

Conclusion: Histone H1 has amphipatic helical regions in its C-terminal region wich become more prominent in the case of spermatozoa. It appears therefore that the interactions found in DNA with helical basic polypeptides (4) might also be present in nucleohistones and protamines. A model for such interactions has been presented elsewhere (5,6)

1) Subirana, J.A. Biopolymers 29: 1351 (1990).
2) Clark, D.J., Hill, C.S., Martin, S.R. and Thomas, J.O. EMBO J. 7: 69 (1988).
3) Subirana, J.A., Muñoz-Guerra, S., Aymamí, J., Radermacher, M. and Frank, J. Chromosoma 91: 377 (1985).
4) Azorín,F., Vives,J., Campos,J.L., Jordán,A., Lloveras,J., Puigjaner,L., Subirana,J.A., Mayer, R. and Brack, A. J.Mol.Biol. 185: 371 (1985).
5) Subirana, J.A. in "Water and Ions in Biomolecular Systems" D.Vasilescu et al., eds., Birkhaüser Verlag AG, Basel, pp 63 (1990).
6) Subirana, J.A. in "Comparative Spermatology 20 Years After" Serono Symposia (1992), in press.

Instituto Juan March (Madrid)

"SCAFFOLD-ATTACHED REGIONS (SAR ELEMENTS): INFLUENCE ON TRANSCRIPTION PREDICTED FROM STRUCTURAL AND BINDING PROPERTIES"

J. Bode[1], T. Kohwi-Shigematsu[2], Y. Kohwi[2], D. Klehr[1], C. Mielke[1] and T. Schlake[1].

[1]Genetik von Eukaryonten, GBF, Gesellschaft für Biotechnologische Forschung mbH, D-3300 Braunschweig, Mascheroder Weg 1.

[2]La Jolla Cancer Res. Found., 10901 North Torrey Pines Rd., La Jolla, CA. 92037.

Scaffold-attached regions (SAR elements) have been identified for a number of eukaryotic genes where they occur in association with established enhancers or as the borders of a chromatin domain. SAR elements themselves share some properties with both the enhancers (transcriptional stimulation independent of distance and orientation) and the locus-control regions (LCRs mediate a copy-number dependent expression and become active only after the integration of a gene construct into genome).

We have investigated the structural features of SAR elements responsible for transcriptional enhancement. For this purpose we have amplified, by oligomerization, the action of a certain core motif common to many SARs. The resulting elements enable an efficient separation of DNA strands under superhelical tension. By this approach artificial elements of a minimum length (175 bp) are created which share both the affinity for the nuclear scaffold and the enhancing properties which are otherwise typical of a SAR comprising 2-3 kb of DNA.

The expression of certain genes is stimulated by millimolar concentrations of butyrate. We have shown that any gene flanked by SAR elements is subject to this effect and conversely that all SAR-specific actions are amplified in the presence of millimolar concentrations of the fatty acid. Among the many activities of butyrate, the hyperacetylation of the nuclear core histones has gained particular attention. Support for this level of action comes from the fact that comparable results have also be obtained with nanomolar amounts of (R)-Trichostatin A, a highly specific inhibitor of histone deacetylases.

Based on their properties, SAR elements have been considered as domain openers. We demonstrate, by inverse PCR techniques, that they create a major target site for the integration of retroviral genomes (10/24 integration sites). In the majority of these cases, the SAR character is located 3' to the retroviral DNA (8/24 sites) but there is no documented case, where a SAR region is hit centrally. Therefore the DNAse I hypersensitive site which is commonly located at the border(s) of a SAR is considered the primary signal for integration.

Instituto Juan March (Madrid)

References

Bode, J., Kohwi, Y., Dickinson, L., Joh, R. T., Klehr, D., Mielke, C., & Kohwi-Shigematsu, T. (1992) Science 255, 195-197.

Klehr, D., Schlake, T., Maass, K. & Bode, J. (1992) Biochemistry 30, in press.

Instituto Juan March (Madrid)

STRUCTURE OF A MULTIMERIC NUCLEOPROTEIN COMPLEX AT THE ORIGINS OF REPLICATION OF BACTERIOPHAGE Ø29.

José M. Hermoso, Manuel Serrano, Raimundo Freire, Crisanto Gutiérrez, and Margarita Salas.
Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma,Canto Blanco, 28049 Madrid, SPAIN.

B. subtilis bacteriophage Ø29 DNA replicates by a protein-priming mechanism. The origins of replication are located at both ends of the linear genome and the initiation reaction is activated by the viral protein p6, due to the formation of a nucleoprotein complex that spans 200-300 bp from the DNA ends.
Protein p6 binding to Ø29 terminal fragments is highly cooperative and induces remarkable changes in the DNA conformation.
Footprint studies revealed protections to hydroxyl radical of 3-4 bp in both strands, across the minor groove, with a periodicity of 12, and DNase I hypersensitivities between contact sites at alternating strands. This pattern suggests that protein p6 binds as a dimer to 24 bp (DNA binding unit) , bending or kinking the DNA every 11-13 bp. In agreement with this, protein p6 restrains positive supercoiling in covalently closed plasmids. These results suggest that the structure of the p6-DNA complex is a right-handed superhelix in which the DNA wraps a protein core.
Concatemers of 9,10, and 12 high affinity binding units were cloned in plasmids and protein p6 binding was confirmed by electron microscopy, using the psoralen technique. Using these constructions we have determined that the change of linking number induced by a single protein p6 dimer (Δk/p6 dimer) was 0.25±0.09. Microccocal nuclease digestion of p6-DNA complexes indicated that the minimal size of the stable complex is about 84 bp. This could be interpreted as the size of one turn of the superhelix stabilized by protein-protein interaction. With these figures we propose a model of the p6 nucleoprotein complex in which the DNA is as packed as in the nucleosome, except that the complex is continuous and the DNA is right-handed.

Instituto Juan March (Madrid)

ROUND TABLE DISCUSSION
PROTEIN-DNA RECOGNITION

R. KAPTEIN
Bijvoet Center for Biomolecular Research
Utrech (The Netherlands)

Instituto Juan March (Madrid)

Round Table Discussion Protein-DNA Recognition

The Round Table was chaired by Dr. A. Travers, who started the discussion by making a few introductory remarks. He stressed the point that although a wealth of structural information on DNA-binding proteins and protein-DNA complexes has become available during the last decade, we still do not understand the basis of the recognition phenomenon. A good predictive theory of sequence specific DNA recognition by proteins is still lacking. A case in point is the FIS protein that was also the subject of the lecture by Dr. W. Saenger. FIS recognizes a number of DNA sequences that have only a weak consensus. It could well be that apart from sequence elements also structural elements are recognized by FIS.

Dr. W. Saenger gave an overview of the various DNA-binding proteins, for which structural information is available, either from X-ray crystallography or from NMR. These proteins range from having little or no sequence specificity, such as the core histones and the enzyme DNase I, which was more fully discussed in the lecture of Dr. D. Suck, to those that have a high binding specificity and usually bind in the major groove of DNA. In the latter class fall a large number of bacterial repressors and the eukaryotic transcription factors, in which there is currently a great deal of interest. These proteins can also be categorized in terms of structural subdomains used for DNA interaction. This leads to families such as helix-turn-helix proteins, leucine zippers, zinc-fingers and so on. Nature has developed a remarkable array of small protein domains to carry out the important task of recognizing target DNA sequences, which is so essential in the regulation of gene expression. Some of these regulatory processes require bending of the DNA and therefore, the subject of DNA-bending induced by proteins such as CAP and FIS has recently attracted a lot of attention. This relates to the question of conformational changes induced either in the DNA or in the protein. It seems that the deformability of in particular B-DNA causes these changes to occur more often in the DNA than in proteins, the strong bending induced by CAP being an extreme example.

Dr. P.B. Sigler continued the discussion on the various physical interactions that are involved in protein-DNA recognition on an atomic level. Apart from the more well known elements such as hydrogen-bonding, Coulomb- and hydrophobic interactions, he stressed the important role played by water molecules in the recognition process. Several water-mediated hydrogen-bonds have been inferred from crystal structures of protein-DNA complexes, the most notable case being that of the Trp repressor complex, where no direct H-bonds are seen between amino acid side chains and essential base-pairs. A lively discussion followed then on this topic, in which also the view from NMR spectroscopy was given. The picture emerging from NMR on water bound to biological macromolecules is somewhat more dynamic than the rather static impression that X-ray crystallography gives. This is due to the fact that NMR often measures rather short residence times (sub-nanosecond) for water molecules seen in specific positions in crystal structures. Nevertheless it is clear that water plays an important role, among various other factors, in the interaction between proteins and nucleic acids. In summary, the discussion illuminated many aspects of protein-DNA recognition, a field of research that is in rapid development.

R. Kaptein
Bijvoet Center for Biomolecular Research, Utrecht

Instituto Juan March (Madrid)

POSTERS

(Other than those presented orally)

Instituto Juan March (Madrid)

THE G-RICH STRAND OF DODECA-SATELLITE, A TANDEM REPEATED DNA SEQUENCE FROM DROSOPHILA MELANOGASTER CENTROMERIC HETEROCHROMATIN, SHOWS UNUSUAL SECONDARY STRUCTURES "IN VITRO".

J.P. Abad, N. Ferrer*, M. Carmena, S. Baars, F. Azorín *and A. Villasante. Centro de Biología Molecular. UAM-CSIC. Cantoblanco. 28049 Madrid.*Dpto. de Biología Molecular y Celular. Centro de Investigación y Desarrollo. CSIC. Barcelona.

Recently we have isolated a new GC-rich satellite from the centromeric heterochromatin of Drosophila melanogaster. This satellite has a repeat unit length of 11-12 bp and has been named dodeca-satellite. Its consensus sequences are CCCGTACTGGT and CCCGTACTCGGT. We have been able to isolated this sequence by screening of a Drosophila melanogaster YAC library with the DNA of a mini-chromosome which contains a functional centromere. This satellite is not very stable in yeast and shows a pattern of instability that might indicate unequal sister chromatid exchanges. Hybridization experiments to DNA from several species of Drosophila, mammals and plants indicate that crosshybridizing sequences are widely distributed. After end labeling of dodeca-satellite fragments (470 bp and 145 bp long) and isolation of both strains, experiments were performed that suggest that the G-rich strand but not the C-rich one is able to form several intramolecular structures. Experiments in progress will tell us the nature of these secondary structures and their possible presence or role "in vivo".

Instituto Juan March (Madrid)

X-Ray Structure Analysis of DNA and RNA Oligonucleotides

Lydia Tabernero, Nuria Verdaguer, Antonio Párraga, Isabel García, Joan Aymamí and Miquel Coll
Unidad de Química Macromolecular. CID-CSIC Diagonal 647, 08028 Barcelona

Two complexes of the DNA dodecamer d(CGCAAATTTGCG) with the minor groove binding drugs Netropsin and Hoechst 33258 have been analyzed by X-ray crystal diffraction. Both complexes crystallize isomorphously with the d(CGCGAATTCGCG) dodecamer and the d(CGCAAATTTGCG)-Distamycin complex. The DNA shows a narrow minor groove conformation with propeller twisted bases in the A-tract as in the Distamicyn complex although they differ in some details. The drug molecules lie in the minor groove in the A-tract being the Hoechst dye clearly disordered in different positions.

Two RNA octamers r(CACAUGUG) and r(UGUGCACA) have been crystallized using $[Co(NH_3)_6]^{3+}$ as counterion. They crystallize in space groups P3 with cell dimensions a = b = 40.85 A c= 27.96 and C2 with a= 49.29 b= 27.09 c= 44.56 respectively. We have collected a first data set up to 3.2 A resolution for the r(CACAUGUG) crystal. An attempt to solve the structure by molecular replacement using an A-RNA model is under way.

Instituto Juan March (Madrid)

BINDING ISOTHERMS FOR DNA-TWO LIGANDS INTERACTION

Colmenarejo, G., Montero, F.

Dpto. de Bioquímica y Biología Molecular I.

Universidad Complutense de Madrid, 28040 Madrid (Spain).

The problem of the simultaneous, competitive and (inter)cooperative interaction of two nonspecific large ligands (i.e. proteins) with DNA is treated using the statistical mechanical method of the Sequence Generating Functions of Lifson.[1] In the most general case (cooperativity and intercooperativity constants different to 1) analitical expressions are not obtained, so the binding isotherm must be numerically computed. In some special situations, analitical expressions are derived: *McGhee-von Hippel binding isotherm* for various ligands (when $\omega_i = 1$), *total saturation binding isotherm* (when $\nu_A/L_A \to 0$, $\nu_B/L_B \to 0$), and *limit crossed isotherm* (when $\nu_A \to 0$). The latter isotherm represents the limit of ν_A/L_A when $\nu_A \to 0$, as a function of ν_B. The total saturation isotherm depends only on n and k (number of base pairs occupied by the ligands), and the limit crossed isotherm depends on all the interaction parameters except K_B and ω_A. This two binding isotherms could be used to estimate some interaction parameters from the proper experimental data.

References.

1. Lifson, S. (1964) *J. Chem. Phys.* **40**,3705-3710.

Instituto Juan March (Madrid)

Lac repressor finds lac operator by intersegment transfer

Reimund Fickert and Benno Müller-Hill

Institut für Genetik der Universität zu Köln,
Weyertal 121, D-5000 Köln 41, FRG

Filterbinding and gel mobility shift assays were used to analyse the kinetics of the interaction of Lac repressor with lac operator. A comparison of the two techniques reveals that filterbinding assays with tetrameric Lac repressor have often been misinterpreted. It has been assumed that all complexes of Lac repressor and lac operator DNA bind with equal affinity to nitrocellulose filters. This assumption is wrong. Sandwich or loop complexes where two lac operators bind to one tetrameric Lac repressor are not retained on nitrocellulose filters. Taking this into account, dimeric and tetrameric Lac repressor do not show any DNA-length dependence of their association and dissociation rate constants when they bind to DNA fragments smaller than 2500 bp carrying a single symmetric ideal lac operator. There is also no evidence for sliding when tetrameric Lac repressor is used in an in vivo titration experiment. A ninefold facilitated location of ideal lac operator by tetrameric but not dimeric Lac repressor on λ DNA is observed. It is presumably due to intersegment transfer involving lac operator-like sequences. This mechanism may play an important role in vivo, too.

Instituto Juan March (Madrid)

CONFORMATIONAL TRANSITION OF POLY(amino^{2}dA-dT)·POLY(amino^{2}dA-dT) INDUCED BY POLYAMINES.

P. Garriga, J. Sági *, D. Garcia-Quintana and J. Manyosa.

Unitat de Biofísica, Departament de Bioquímica i de Biologia Molecular, Universitat Autònoma de Barcelona, Spain.
** Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.*

Poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) has been the subject of recent studies undertaken to investigate the role of the amino exocyclic group in its conformational isomerizations. The conformation adopted by this polynucleotide in high-salt solutions has been the matter of some controversy. Previous studies proposed poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) in NaCl-containing solution (3.5 M) to be in a Z-conformation (1). Later, NMR studies were interpreted as reflecting an A-conformation (2). However, this interpretation was questioned by other authors who claimed that, in these conditions, the polynucleotide adopted the novel type of helix called X-DNA (3,4) which could be stabilized with submillimolar concentrations of Mg^{2+}. Nevertheless, our latest FTIR results obtained with poly(amino^{2}dA-dT)· poly(amino^{2}dA-dT) in Mg^{2+}-containing solution and in films of low relative humidity (5), are highly compatible with an A-conformation for the polynucleotide in agreement with the previously cited NMR study (2).

In order to further investigate the structural features of this polynucleotide, its interaction with spermine and spermidine has been studied. These naturally occurring polyamines are implicated in a variety of cellular reactions (6). They have been reported to induce the B-to-Z transition in several cases (7,8). Interestingly, recent molecular dynamics of spermine-DNA interactions have provided further evidence that the binding of polyamines to DNA can be sequence specific (9).

Instituto Juan March (Madrid)

We have used absorption and fourth derivative UV (4), FTIR and CD spectroscopies to study the effect of spermine and spermidine on the conformational properties of poly(amino^{2}dA-dT)· poly(amino^{2}dA-dT). At low ionic strength and neutral pH a conformational transition is induced with 30 μM spermine. Thermal denaturation of the spermine-polynucleotide complex is a cooperative process with a t_m of 94.5°C. An analogous behavior is observed in the case of spermidine. The results reported show that spermine and spermidine induce the same conformational transition as the previously obtained using Mg^{2+} and in dehydrated films (5).

REFERENCES

(1) Howard, F.B., Chen, C.W., Cohen, J.S., and Miles, H.T. (1984) *Biochem. Biophys. Res. Commun. 118,* 848-853.

(2) Borah, B., Howard, F.B., Miles, H.T. and Cohen, J.S. (1986) *Biochemistry 25,* 7464-7470.

(3) Vorlickova, M., Sági, J., Szabolcs, A., Szemzo, A., Otvos, L. and Kypr, J. (1988) *Nucleic Acids Res. 16,* 279-289.

(4) Garriga, P., Sági, J., Sabés, M., Garcia-Quintana, D. and Manyosa, J. (1990) *J. Biomol. Struct. Dyn. 7,* 1061-1071.

(5) Garriga, P., Sági, J., Garcia-Quintana D. and Manyosa, J. (1992) submitted.

(6) Tabor, C.W. and Tabor, H. (1984) *Ann. Rev. Biochem. 53,* 749-790.

(7) Behe, M. and Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. USA 78,* 1619-1623.

(8) Thomas, T.J. and Messner, R.P. (1986) *Nucleic Acids Res. 14,* 6721-6733.

(9) Feuerstein, B.G., Pattabiraman, N. and Marton, L.J. (1989) *Nucleic Acids Res. 17,* 6883-6892.

Instituto Juan March (Madrid)

MUTANT GCN4 PROTEINS WITH ALTERED DNA-BINDING SPECIFICITY

J. Kim, D. Tzamarias & K. Struhl. Harvard Medical School, Department of Biological Chemistry & Molecular Pharmacology, 240 Longwood Avenue, Boston, MA 02115.

The yeast GCN4 protein is the transcriptional activator of many amino acid biosynthetic genes, including His3. This protein binds the same sequence, ATGACTCAT, as Jun-Fos oncoproteins *via* a bZIP domain consisting of a leucine zipper dimerization element and an adjacent basic region that directly contacts DNA.

It has been shown that a dimerized peptide consisting of the basic region alone is sufficient for specific DNA binding. However, the contribution of each specific amino acid to the binding specificity is largely unknown. To investigate this issue, a set of yeast strains were made which contained a series of mutant His3 promoters differing only in their GCN4 recognition sequences, upstream of the TATA element. To isolate potential altered specificity mutants, a collection of GCN4 proteins generated by mutagenesis *in vitro* with degenerate oligonucleotides, was screened *in vivo* for their ability to activate His3 transcription from the symmetrically mutated target sequences.

Single mutant proteins, 235Trp and 242Ser were isolated which activate transcription at the altered binding sites **T**TGACTCA**A** and AT**T**ACT**A**AT respectively which do not allow activation by wild type protein. One double mutant which contains 242Ser to Cys and 246Lys to Gln substitutes can bind to the site ,AT**T**ACT**A**AT, *in vitro* and activate His3 transcription *in vivo*. Interestingly, each of the single mutations binds *in vitro* but fail to activate *in vivo*.

Double mutant protein, 235Trp- 242Ser, and triple mutant protein, 235Trp-242Cys-246Gln, bind to the doubly mutated site of **T**T**T**ACT**A**A**A** .

Therefore, we report here that 235Trp and 242Ser are crucial residues for the recognition of A and T bases, G and C bases of **A**T**G**ACT**C**A**T** sequence respectively and there might be an interaction of 242Ser and 246Lys residues for the recognition of DNA sequence.

Instituto Juan March (Madrid)

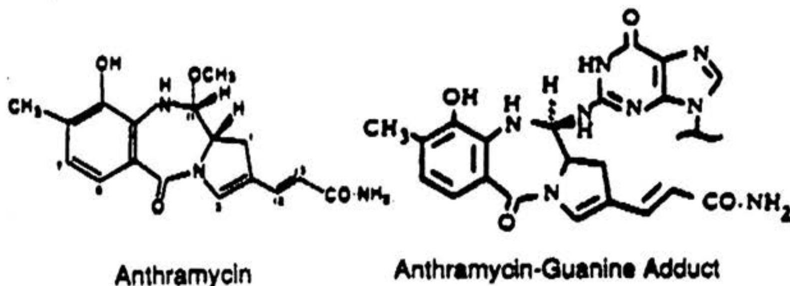
THE CRYSTAL STRUCTURE OF A COVALENT DNA ADDUCT: ANTHRAMYCIN BOUND TO CCAACGTTGG

Mary L. Kopka, Andrei Lipanov, Kazimierz Grzeskowiak and Richard E. Dickerson
Molecular Biology Inst., University of California at Los Angeles, Los Angeles, CA 90024, USA

Anthramycin, an anti-tumor antibiotic that binds covalently to DNA (Fig 1.), has been covalently bound to the oligomer, CCAACGTTGG, and crystallized. The chiral C-11 atom of anthramycin forms a covalent bond with guanine N2 (Fig. 1). Two adducts were separated by HPLC on a C-18 column and each was crystallized. However, further purification was needed, and ADDUCT 1 went through a second HPLC purification, while ADDUCT 2 had three HPLC runs. Both adducts crystallized in the trigonal space group P3(1)21 and data sets were collected on a Rigaku RAXIS IIC Image Plate, with ADDUCT 1 data collected to 3.0 Å and ADDUCT 2 collected to 2.2 Å.

Refinement of ADDUCT 1 has been completed and ADDUCT 2 refinement is continuing. The drug has the possibility of attaching at two places on the helix — at one of the guanines on the ends of the sequence or to a guanine in the center of the sequence. The ADDUCT 2 cell volume of 46,100 Å^3 suggests one-half double helical DNA decamer per assymetric unit (3 per cell) in a crystal 46% DNA by weight. The decamer helix must sit on a special position. Its two ends related by a twofold symmetry axis. It appears that an anthramycin molecule is bound to each end of ADDUCT 2. This is not the case for ADDUCT 1, cell volume of 94,500 Å^3 with one molecule per assymetric unit (6 per cell), which could have either one or two drug molecules bound. The full structure analysis of each adduct will elucidate how the drug binds, as well as clarify how many drug molecules are bound per double helix.

Figure 1



Instituto Juan March (Madrid)

DNA TOPOLOGY IN HALOPHILIC ARCHAEBACTERIA

F.J.M.Mojica, F.Charbonnier and P.Forterre.

Institut de Génétique et Microbiologie. Université Paris-Sud, Centre D'Orsay Cedex. France.

At the molecular level, archaebacteria exhibit a mixture of eubacterial and eukaryotic features as well as unique ones. Since the mechanisms which control DNA topology are drastically different in eubacteria and eukaryotes, it is interesting to investigate them in archaebacteria. It has been shown previously that many features of DNA topology in halophilic archaebacteria are of the eubacterial-type: presence of a type II DNA topoisomerase homologous to eubacterial DNA gyrase, negatively supercoiled plasmids, absence of a DNA topoisomerase relaxing positive superturns. In order to determine if this similarity extends to the influence of the environment on the level of DNA superhelicity, we have investigated the effect of salt and temperature on plasmid supercoiling in *Haloferax volcanii*. In eubacteria, an increase in salt concentration (1) or temperature (2) induces an increase en negative supercoiling. This has been interpreted as a evidence of a role of supercoiling in gene regulation (1) and for a constant value for supercoiling at different temperatures (2). In contrast to these results, we have found that an increase in salt concentration has only a slight effect on DNA supercoiling of the *Haloferax volcanii* plasmid PHV11, whereas an increase in temperature results in a decrease of the negative superhelicity of the pHV11 plasmid. These results indicate that the observation on DNA topological control made with *E.coli* cannot be extrapolated to all prokaryotic microorganisms.

REFERENCES

(1) L-S. Hsieh, J. Rouviere-Yaniv and K. Drlica. 1991. Bacterial DNA Supercoiling and [ATP]/[ADP] Ratio: Changes Associated with Salt Shock. J.Bacteriol. **173**: 3914-3917.

(2) E. Goldstein and K. Drlica. 1984. Regulation of Bacterial DNA supercoiling: Plasmid linking numbers vary with growth temperature. Proc. Natl. Acad. Sci. USA. **81**: 4046-4050.

Instituto Juan March (Madrid)

FUNCTIONAL SIGNIFICANCE OF A STABLE CRUCIFORM AT THE 3' END OF THE YEAST *FBP1* GENE.

Marcel·lí del Olmo, Agustín Aranda, Luis Franco and José E. Pérez-Ortín
Departamento de Bioquímica y Biología Molecular. Facultades de Ciencias. Universitat de València. Spain.

A DNA fragment with an inverted repeat can adopt two possible stable conformations: the double helix or a structure with two stems and loops which has been called cruciform. The formation of cruciforms is energetically not favourable so they can only appear in supercoiled DNA. Once formed, this structure can be specifically cleaved by several endonucleases either in the unpaired bases at the loop (e.g., S1 nuclease) or in the cruciform-B DNA junctions (e.g., endonuclease VII of T4 bacteriophage). Inverted repeats potentially able to form cruciforms have been found in many different organisms and sites, specially in promoters and regions of transcription termination in bacteria and viruses. This preferential location has been interpreted as a putative implication of these sequences in the regulation of the gene expression. The existence of cruciforms in superhelical DNA in vitro has been demonstrated in many cases using the above indicated nucleases and other methods. The existence of cruciforms in vivo is less certain. The first evidence about the presence of cruciforms in vivo in prokaryotes was obtained with $(dA\text{-}dT)_n$ tracts (1). More recently other groups have arrived to the same conclusion and it has also been shown that cruciform extrusion depends on the environmental conditions (2). The existence of cruciforms in eukaryotes is not well documented although it has been demonstrated that artificial $(dA\text{-}dT)_n$ tracts can exist as cruciforms *in vivo* in reconstituted chromatin in *Xenopus* oocytes (3).

We have detected that an imperfect $(dA\text{-}dT)_{12}$ tract placed at the end of the *FBP1* gene is part of a site hypersensitive to several nucleases in chromatin (4) which acts, probably, as a boundary positioning nucleosomes at both sides. Its S1 nuclease sensitivity in naked DNA depends on the supercoiling state and it is cut by the cruciform-specific T4 endonuclease VII. Nucleotide-precision mapping of the S1 and endo VII cutting sites and topoisomer distribution in $(dA\text{-}dT)_{12}$-containing plasmids demonstrates the existence of the predicted cruciform. Using S1 3' end mapping and a recently developed functional assay (5) we have found that this site acts as a transcriptional terminator what rises the possibility of a functional implication of the cruciform structure in DNA or the concomitant mRNA hairpin in the process of transcriptional termination in yeast.

1.-Haniford D.B. and Pulleyblank D. E. (1985) Nucleic Acids Res. 13, 4343-4363.
2.-Dayn A. et al., (1991) J. Bacteriol. 173, 2658-2664.
3.- Leonard, M.W. and Patient, R.K. (1991). Mol. Cell. Biol. 11, 6128-6138.
4.- Del Olmo. M., Sogo, J.M., Franco, L. and Pérez-Ortín, J.E. (in preparation).
5.- Irniger, S. Egli, C.M. and Braus, G.H. (1991). Mol. Cell. Bio. 11 3060-3069.

Instituto Juan March (Madrid)

DEOXYRIBOENDONUCLEASES FROM STREPTOMYCES WHICH DO NOT BELONG TO RESTRICTION-MODIFICATION SYSTEMS

Jesús Sánchez, Santiago Cal y Jesús F. Aparicio
Area de Microbiología, Departamento de Biología Funcional
Facultad de Medicina, Oviedo, Spain.

Looking for restriction-modification systems with new specifities in *Streptomyces*, we detected several DNAses which do not belong to those systems and which had not been studied previously, due to the fact that they conceivably play a general nutritional role and also to their supposed non-specificity, which would not permit their application to the restriction analysis of DNA. When we studied these DNAses in more detail, they showed however some interesting characteristics, as were their circumstantial role in in-vivo phage restriction and their periplasmic location which explain the lack of a corresponding modification activity (1,2). In some instances, as in the *S. antibioticus* DNAse, the enzyme shows a specific pattern of bands in agarose gels after the hydrolysis of several DNA substrates, whereas in others (as in *S. glaucescens*) the enzyme is apparently unspecific. The secretion of these DNAses temporally precedes the cellular differentiation (aerial mycelium formation), both processes being controlled by analogous nutritional conditions. Both enzymes (*S. glaucescens* and *S. antibioticus* DNAses) have been purified and their main biochemical characteristics studied (3,4). The two DNAses produced nicks in a chain of double stranded DNA and generated 3'-OH and 5'-P termini, resulting in the production of double-stranded fragments with 5' and/or 3' - protruding single-stranded tails. The analysis of the terminal nucleotides of the generated fragments and the sequencing of partially digested DNA showed a different cleavage specificity for both enzymes. Whereas the *S. glaucescens* DNAse cleaves double stranded DNA preferably at the dinucleotide step 5'-CpC-3', the *S. antibioticus* enzyme seems to extend the specificity to at least a trinucleotide sequence, being 5'-GpGpG-3' one of those more frequently cut (4,5). Other DNA specificities of a structural type suggested by the behaviour of these enzymes, are presently being analyzed.

(1) De los Reyes, Gavilán C.G. et al (1988). J. Bacteriol. *170*: 1339-1345.

(2) Aparicio, J.F. et al. (1988) J. Gen. Microbiol. *134*: 2345-2351.

(3) Aparicio, J.F. et al. (1992). Biochem. J. *281*: 231-237.

(4) Aparicio, J.F. et al. (1992). Eur. J. Biochem. in press.

(5) Cal, S. and J. Sánchez, unpublished results.

Instituto Juan March (Madrid)

BACTERIOPHAGE Ø29 dsDNA-BINDING PROTEIN: FEATURES AT THE REPLICATION ORIGINS REQUIRED TO ACTIVATE THE INITIATION OF REPLICATION AND PROTEIN MOTIF INVOLVED IN DNA BINDING

Manuel Serrano, Raimundo Freire, Margarita Salas and José M. Hermoso.
Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Cantoblanco, 28049 Madrid, Spain.

Bacillus subtilis phage Ø29 has a linear double-stranded DNA with a terminal protein covalently bound at both 5´-ends. Replication is initiated at both genome ends by the viral DNA polymerase using the terminal protein as primer. The Ø29 dsDNA-binding protein (p6) is required *in vivo* for Ø29 DNA replication and activates the initiation of replication in an *in vitro* system with purified proteins by forming a multimeric nucleoprotein complex at the replication origins; the basic unit of binding is a protein p6 dimer bound to 24 bp.

By studying insertion mutant replication origins it was concluded that the phasing of the protein p6 multimer with respect to the genome end is essential and, therefore, the only mutants that were activated by protein p6 were those with a 24 bp insertion. The presence of a poly-pirimidine tract of 7 nucleotides conserved at both replication origins suggests that it could play an important role in the activation process. A mutant replication origin with a 24 bp deletion that includes the poly-pirimidine tract was not activated by protein p6; since this region is not required for protein p6 binding we tentatively propose that it could act as a protein p6-dependent DNA unwinding element. In addition, protein p6 activation of initiation is higher at lower temperatures in agreement with the hypothesis that protein p6 is favouring the unwinding of DNA at the replication origins.

The main DNA recognition regions for protein p6 have been mapped at each replication origin and DNA bendability predictions indicate that they contain regions bendable every 12 bp. A DNA concatemer formed by direct repeats of a bendable binding unit (24 bp) present in one of these recognition regions has been constructed and cloned. DNaseI footprints indicate that protein p6 binds to these sequences in the same position and with higher affinity than to the replication origin.

The analysis of amino-terminus deletion mutants suggests that the first 13 amino acid residues are important for DNA binding; this region is predicted to form an amphipatic α-helix structure. A model is proposed in which this α-helix interacts with the DNA minor groove through the underlined amino acid residues: 1-AKMMQREITKTTVN -14. We have carried out site directed mutagenesis in this region and several mutant proteins have been purified and studied. The R6A mutant was completely unable to bind DNA and to activate the initiation of replication while mutant K2A was partially active in both assays.

Instituto Juan March (Madrid)

B_h-DNA: Ionic-Water Interactions require Ade in Napoly[d(A)]:poly[d(T)] to be tilted about 12º, the Thy tilt being about 0.

M.E. Rikhirev, D.B. Beglov and I. Ya. Skuratovskii
Institute of Molecular Genetics, Moscow

Recently Lipanov et al. (*J. Biomol. Struct. Dyn* 7, 811 (1990)) have shown an AT base pair to be buckled about 12º in the Na-poly[d(A)]:poly[d(T)] in fibres. However the A-tilted:T-not or A-not:T-tilted situation was uncertain. A molecular graphics study combined with the "energy" optimization of Na-$(H_2O)_6$ octahedra allowing for replacing H_2O molecules for the O and N of the DNA has been done.

A sterically reasonable variants of Na-$(H_2O)_n$-n<6 fitting in either the minor or major grooves of Na-poly[d(A)]:poly[d(T)] clearly prefer the A-tilted:T-not variant over another one, the agreement with the original X-ray diffraction data set (Arnott et al., *Nucl.Acids Res* 11, 2,4141 (1983)) being improved essentally. Intermolecular via-H_2O-H-bonding in a crystal is as well probable to stabilize the A buckled structure of Na-poly[d(A)]:poly[d(T)]. Either the steric or diffraction aspects of the structure require Na+ ions to be situated at the very bottom of the minor groove.

Instituto Juan March (Madrid)

X-ray fibre diffraction: X-form DNA = A-form DNA !?

D.A.Konyukhov[1], L.I.Volkova[1], D.G.Alexeev[1], N.M.Akimenko[1], J.Sagi[2], M.Vorlickova[3], J.Kypr[3] and I.Ya.Skuratovskii[1]

[1]Institute of Molecular Genetics, Moscow
[2]Central Research Institute of Chemistry, Budapest
[3]Institute of Biophysics, Brno

Z-form like CD spectra of poly[d(amino^{2}AT)]:poly[d(amino^{2}AT)] in 40 to 50% ethanol or in 1.0 to 2.5 M NaCl (Vorlickova M. et al., *Nucl. Acids Res.*, **16**, 279 (1988)) are the spectra of the X-form DNA adopted by poly[d(AT)]:poly[d(AT)] at very high CsF concentrations (Vorlicova M. et al., J. *Mol. Biol.*, **166**, 85 (1983)), the am^{2}A:T base-pair geometry being intriguing when compared to that of the G:me^{5}C.

A newly synthesized and well purified Na-poly[d(am^{2}AT)]:poly[d(am^{2}AT)] material yields in fibres a classical A-form DNA pattern within the 44 to 98% rel. hum. range. Previosly a not so well purified material of a rather small and heterogeneous polynucleotide length (0.2 to 1.0 kb) yielded the A-like diffraction patterns which were not too good to get the molecular and crystal structure parameters unambiguously, those patterns yet implying that the structure is stable against the significant changes in the added NaCl or $MgCl_2$ content (Alexeev D. et al., *Stud. Biophys.*, **136**, 189, (1990)).

Instituto Juan March (Madrid)

LIST OF INVITED SPEAKERS

Instituto Juan March (Madrid)

Workshop on

DNA STRUCTURE AND PROTEIN RECOGNITION

List of Invited Speakers

F. Azorín	*Departamento de Biología Molecular, Centro de Investigación y Desarrollo, C.S.I.C., Jordi Girona Salgado, 18-26, 08034 Barcelona (Spain).* *Tel.: 34 3 204 06 00 Ext. 317* *Fax : 34 3 204 59 04*
D.M. Crothers	*Department of Chemistry, Yale University, P.O. Box 6666, New Haven, CT. 06511 (USA).* *Tel.: [1] (203) 432 3916* *Fax : [1] (203) 432 6144*
R.E. Dickerson	*Molecular Biology Institute, University of California, Los Angeles, CA. 90024 (USA).* *Tel.: [1] (213) 825 5864* *Fax : [1] (213) 825 0982*
M. D. Frank-Kamenetskii	*Institute of Molecular Genetics, Russian Academy of Sciences, Moscow 123182 (Russia).* *Tel.: [70] (95) 1962075* *Fax : [70] (95) 1960221*
C.W. Hilbers	*Nijmegen SON Research Center, Laboratory of Biophysical Chemistry, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen (The Netherlands).* *Tel.: 31 80 652160* *Fax : 31 80 652112*
R. Kaptein	*Bijvoet Center for Biomolecular Research, University of Utrecht, Padualaan 8, 3584 CH Utrecht (The Netherlands).* *Tel.: [31] (30) 53 2184* *Fax : [31] (30) 54 0980*
A. Klug	*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH (U.K.).* *Tel.: [44] (223) 402253* *Fax : [44] (223) 412231*
L. Kohlstaedt	*Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, 260 Whitney Avenue, P.O. Box 6666, New Haven, CT. 06511 (USA).* *Tel.: [1] (203) 432-5617* *Fax : [1] (203) 432 3282*

Instituto Juan March (Madrid)

D. Moras	*Laboratoire de Cristallographie Biologique, IBMC, Centre National de la Recherche Scientifique, 15,rue Descartes, 67084 Strasbourg Cedex (France).* *Tel.: [33] (88) 610202 x 381* *Fax : [33] (88) 607550*
D. Rhodes	*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH (U.K.).* *Tel.: [44] (223) 248011 Ext. 2441* *Fax : [44] (223) 213556*
W. Saenger	*Institut für Kristallographie der FU, Takustrasse 6, 1000 Berlin 33, (Germany).* *Tel.: [49] (30) 8383412* *Fax : [49] (30) 8326561*
M. Salas	*Centro de Biología Molecular, C.S.I.C. Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).* *Tel.: (34-1) 397 50 70* *Fax : (34-1) 397 47 99*
P.B. Sigler	*Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, 260 Whitney Avenue, New Haven, CT. 06511 (USA).* *Tel.: [1] (203) 737 4442* *Fax : [1] (203) 776 3550*
J.A. Subirana	*Departamento de Ingeniería Química UPC, E.T.S.I. Industriales, Avda. Diagonal 647, 08028 Barcelona (Spain).* *Tel.: (34-3) 401 66 88* *Fax : (34-3) 401 66 00*
D. Suck	*European Molecular Biology Laboratory, Postfach 102209, Meyerhofstrasse 1, D-6900 Heidelberg (Germany).* *Tel.: [49] 6221-387307* *Fax : [49] 6221-387306*
A. Travers	*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.)* *Tel.: [44] (223) 402 253* *Fax : [44] (223) 412 231*
J.C. Wang	*Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA. 02138 (USA).* *Tel.: [1] (617) 495-1901* *Fax : [1] (617) 495-0758*

Instituto Juan March (Madrid)

Workshop on

DNA STRUCTURE AND PROTEIN RECOGNITION

List of Participants

J.P. Abad
Centro de Biología Molecular, C.S.I.C., Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 41 80
Fax : 34 1 397 47 99

J. Bode
GBF Gesellschaft für Biotecnologische Forschung m.b.H., Genetik von Eukaryonten, Mascheroder Weg 1, D-3300 Braunschweig-Stöckheim (Germany)
Fax : 49 531 618 1515

J.L. Campos
Unidad de Fisiología Vegetal, Facultad de Farmacia, Universidad de Barcelona, 08028 Barcelona (Spain)
Tel.: 34 3 330 9017

P. Carrera
Departamento de Biología Molecular y Celular, Centro de Investigación y Desarrollo, C.S.I.C., Jordi Girona, 18-26, 08034 Barcelona (Spain).
Tel.: 34 3 204 06 00
Fax : 34 3 204 59 04

J.L. Castrillo
Centro de Biología Molecular, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain)
Te.: 34 1 397 84 51
Fax : 34 1 397 47 99

M. Coll
Unidad de Química Macromolecular, C.S.I.C.- E.T.S.I. Industriales, Diagonal 647, 08028 Barcelona (Spain),
Tel.: 34 3 401 6687
Fax : 34 3 401 66 00

G. Colmenarejo
Departamento de Bioquímica y Biología Molecular I. Facultad de Ciencias, Universidad Complutense, Ciudad Universitaria, 28040 Madrid (Spain).
Tel.: 34 1 394 41 58
Fax : 34 1 394 41 59

J.R. Daban
Departamento de Bioquímica y de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Barcelona, Edificio C. 08193 Bellaterra, Barcelona (Spain).
Tel.: 34 3 581 12 64
Fax : 34 3 581 20 03

Instituto Juan March (Madrid)

X. Fernández-Busquets	*Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Barcelona, Edificio C, 08193 Bellaterra, Barcelona (Spain).* *Tel.: 34 3 581 12 64* *Fax : 34 3 581 20 03*
R. Fickert	*Institut für Genetik, Universität zu Köln, Weyertal 121, D-5000 Köln 41 (Germany).* *Tel.: 49 221 470 34 07* *Fax : 49 221 470 51 70*
F. Gago	*Departamento de Fisiología y Farmacología, Universidad de Alcalá de Henares, Campus Universitario, Carretera Madrid-Barcelona, Km. 33,600, 28871 Alcalá de Henares, Madrid (Spain).* *Tel.: 34 1 885 45 14* *Fax : 34 1 885 45 44*
M. García-Mateu	*Centro de Biología Molecular, C.S.I.C., Facultad de Ciencias. Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).* *Tel.: 34 1 397 50 70* *Fax : 34 1 397 47 99*
P. Garriga	*Departamento de Bioquímica y Biología Molecular, Unidad de Biofísica, Universidad Autónoma de Barcelona, Edificio M, 08193 Bellaterra, Barcelona (Spain).* *Tel.: 34 3 581 14 75* *Fax : 34 3 581 20 04*
R. Giraldo	*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.)* *Tel.: 44 223 248011 Ext. 2295* *Fax : 44 223 213556*
J.M. Hermoso	*Centro de Biología Molecular, C.S.I.C., Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain).* *Tel.: 34 1 397 84 29* *Fax : 34 1 397 47 99*
A. Jacobo-Molina	*Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ. 08854-5638 (USA).* *Tel.: 908 463 4343* *Fax : 908 463 5318*

Instituto Juan March (Madrid)

J. Kim	*Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA. 02115 (USA).* *Tel.: 617 432 3103* *Fax : 617 432 2529*
M.L. Kopka	*UCLA DNA Structure Group, Molecular Biology Institute, University of California, 405 Hilgard Avenue, Los Angeles, CA. 90024-1570 (USA).* *Tel.: 310 206 8278* *Fax : 310 825 0982*
R.F. Macaya	*UCLA DNA Structure Group, Chemistry and Biochemistry Department, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA. 90024-1569 (USA).* *Tel.: 310 825 9232* *Fax : 310 825 0982*
F.J. M. Mojica	*Departamento de Genética Molecular y Microbiología. Universidad de Alicante, Campus de San Juan, Apartado 374, 03080 Alicante (Spain)* *Tel.: 34 6 565 85 54* *Fax : 34 6 565 85 57*
J. Pérez-Martín	*Centro de Investigaciones Biológicas, C.S.I.C., Lab. del Dr. de Lorenzo, Velázquez 144, 28006 Madrid (Spain).* *Tel.: 34 1 561 18 00* *Fax : 34 1 562 75 18*
J.E. Pérez-Ortín	*Departamento de Bioquímica y Biología Molecular. Facultad de Ciencias, Universidad de Valencia, Dr. Moliner, 50, 46100 Burjassot, Valencia (Spain).* *Tel.: 34 6 386 4385* *Fax : 34 6 386 4372*
W.L. Peticolas	*Department of Chemistry, College of Arts and Sciences, University of Oregon, Eugene, OR. 97403-1253 (USA)* *Tel.: 503 346 4601* *Fax : 503 346 4643*
J. Sánchez	*Departamento de Biología Funcional, Area de Microbiología, Facultad de Medicina, Universidad de Oviedo, c/Julián Clavería, s/nº, 33071 Oviedo (Spain).* *Tel.: 34 85 10 35 27* *Fax : 34 85 23 22 55*

Instituto Juan March (Madrid)

M. Sanderson

Cancer Research Campaign, CRC Biomolecular Structure Unit, The Institute of Cancer Research, (University of London), Cotswold Road, Sutton, Surrey SM2 5NG (U.K.).
Tel.: 081 643 8901
Fax : 081 770 7893

J.W.R. Schwabe

MRC, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH (U.K.).
Tel.: 0223 402 444
Fax : 0223 213 556

M. Serrano

Centro de Biología Molecular, C.S.I.C., Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

Z. Shakked

Department of Structural Biology, The Weizmann Institute of Science, Rehovot 76100 (Israel).
Tel.: 972 8 342 672
Fax : 972 8 473 039

I.Y. Skuratovskii

Institute of Molecular Genetics of the Russian Academy of Sciences, Kurchatov Sq., 46, 123182 Moscow (Russia)
Fax : 095 196 0221

A. Stasiak

Laboratoire d'Analyse Ultrastructurale, Bâtiment de Biologie, Niveau 1 Université de Lausanne, CH-1015 Lausanne-Dorigny (Switzerland).
Tel.: 4121 692 24 71
Fax : 4121 692 25 40

N. Verdaguer

Departamento de Ingeniería Quimica UPC, E.T.S.I. Industriales, Avda. Diagonal 647, 08028 Barcelona (Spain).
Tel.: 34 3 401 66 88
Fax : 34 3 401 66 00

M. Wolters

Freie Universität Berlin, Institut für Molekularbiologie und Biochemie, Bereich Molekularbiologie UND Informatik, Arnimallee 22, D-1000 Berlin 33 (Germany).
Tel.: 49 30 838 25 27
Fax : 49 30 838 48 02

Instituto Juan March (Madrid)

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

246 **Workshop on Tolerance: Mechanisms and implications.**
Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
Course on DNA - Protein Interaction.

249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
Organized by P. Puigdoménech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdoménech and M. Pagès.

252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening, J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L Calderón.

255 **Workshop on The Reference Points in Evolution.**
Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P Wagner, D. B. Wake and A. Wilson.

256 **Workshop on Chromatin Structure and Gene Expression.**
Organized by F. Azorín, M. Beato and A. A. Travers. Lectures by F. Azorín, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carrillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrange and C. Wu.

Instituto Juan March (Madrid)

257 **Lecture Course on Polyamines as modulators of Plant Development.**
Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

258 **Workshop on Flower Development.**
Organized by H. Saedler, J. P. Beltrán and J. Paz Ares. Lectures by P. Albersheim, J. P. Beltrán, E. Coen, G. W. Haughn, J. Leemans, E. Lifschitz, C. Martin, J. M. Martínez-Zapater, E. M. Meyerowitz, J. Paz-Ares, H. Saedler, C. P. Scutt, H. Sommer, R. D. Thompson and K. Tran Thahn Van.

259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

260 **Lecture Course Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organized by T. Ruiz-Argüeso. Lectures by T. Bisseling, P. Boistard, J. A. Downie, D. W. Emerich, J. Kijne, J. Olivares, T. Ruiz-Argüeso, F. Sánchez and H. P. Spaink.

261 **Workshop The Regulation of Translation in Animal Virus-Infected Cells.**
Organized by N. Sonenberg and L. Carrasco. Lectures by V. Agol, R. Bablanian, L. Carrasco , M. J. Clemens, E. Ehrenfeld, D. Etchison, R. F. Garry, J. W. B. Hershey, A. G. Hovanessian, R. J. Jackson, M. G. Katze, M. B. Mathews, W. C. Merrick, D. J. Rowlands, P. Sarnow, R. J. Schneider, A. J. Shatkin, N. Sonenberg, H. O. Voorma and E. Wimmer.

263 **Lecture Course on the Polymerase Chain Reaction.**
Organized by M. Perucho and E. Martínez-Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. Mc Clelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 **Workshop on Yeast Transport and Energetics.**
Organized by A. Rodríguez-Navarro and R. Lagunas. Lectures by M. R. Chevallier, A. A. Eddy, Y. Eilam, G. F. Fuhrmann, A. Goffeau, M. Höfer, A. Kotyk, D. Kuschmitz, R. Lagunas, C. Leão, L. A. Okorokov, A. Peña, J. Ramos, A. Rodríguez-Navarro, W. A. Scheffers and J. M. Thevelein

265 **Workshop on Adhesion Receptors in the Immune System.**
Organized by T. A. Springer and F. Sánchez-Madrid. Lectures by S. J. Burakoff, A. L. Corbi-López, C. Figdor, B. Furie, J. C. Gutiérrez-Ramos, A. Hamann, N. Hogg, L. Lasky, R. R. Lobb, J. A. López de Castro, B. Malissen, P. Moingeon, K. Okumura, J. C. Paulson, F. Sánchez-Madrid, S. Shaw, T. A. Springer, T. F. Tedder and A. F. Williams.

266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
Organized by F. X. Avilés. Lectures by T. L. Blundell, W. Bode, P. Carbonero, R. W.Carrell, C. S. Craik, T. E. Creighton, E. W. Davie, L. D. Fricker, H. Fritz, R. Huber, J. Kenny, H. Neurath, A. Puigserver, C. A. Ryan, J. J. Sánchez-Serrano, S. Shaltiel, R. L. Stevens, K. Suzuki, V. Turk, J. Vendrell and K. Wüthrich.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organized by J. M. Mato and J. Larner. Lectures by M. V. Chao, R. V. Farese, J. E. Felíu, G. N. Gaulton, H. U. Häring, C. Jacquemin, J. Larner, M. G. Low, M. Martín Lomas, J. M. Mato, E. Rodríguez-Boulan, G. Romero, G. Rougon, A. R. Saltiel, P. Strålfors and I. Varela-Nieto.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organized by R. Serrano and J. A. Pintor-

Instituto Juan March (Madrid)

Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organized by R. Baker and J. M. Delgado-García. Lectures by C. Acuña, R. Baker, A. H. Bass, A. Berthoz, A. L. Bianchi, J. R. Bloedel, W. Buño, R. E. Burke, R. Caminiti, G. Cheron, J. M. Delgado-García, E. E. Fetz, R. Gallego, S. Grillner, D. Guitton, S. M. Highstein, F. Mora, F. J. Rubia Vila, Y. Shinoda, M. Steriade and P. L. Strick.

Texts published by the CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organized by C. Belmonte and F. Cerveró. Lectures by C. Belmonte, G. J. Bennet, J. N. Campbell, F. Cerveró, A. W. Duggan, J. Gallar, H. O. Handwerker, M. Koltzenburg, R. H. LaMotte, R. A. Meyer, J. Ochoa, E. R. Perl, H. P. Rang, P. W. Reeh, H. G. Schaible, R. F. Schmidt, J. Szolcsányi, E. Torebjörk, W. D. Willis Jr.

Instituto Juan March (Madrid)

The Centre for International Meetings on Biology
has been created within the
Instituto Juan March de Estudios e Investigaciones,
a private foundation which complements the work
of the *Fundación Juan March* (established in 1955)
as an entity specialized in scientific activities
in general.

The Centre's initiatives stem from the Plan
for International Meetings on Biology,
supported by the *Fundación Juan March*.
A total of 30 meetings and 3 Juan March Lecture
Cycles, all dealing with a wide range of subjects
of biological interest, were organized between
1989 and 1991 within the scope of this Plan.

The Centre endeavours to actively and
sistematically promote cooperation among Spanish
and foreign scientists working in the field of Biology,
through the organization of Lecture
and Experimental Courses, Workshops, Seminars,
Symposia and the Juan March Lectures on Biology.

Instituto Juan March (Madrid)

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • Teléfono 34 - 1 - 435 42 40 • Fax 576 34 20
28006 Madrid (España)

The lectures summarized in this publication were presented by their authors at a workshop held on the 16th through the 18th of March, 1992, at the Instituto Juan March.

All published articles are exact reproductions of author's text.

There is a limited edition of 350 copies of this volume, available free of charge.