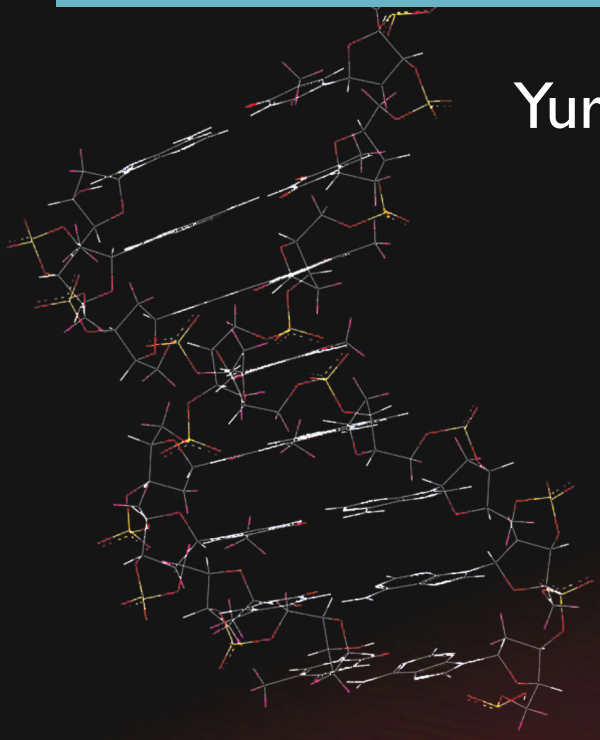
THE LIQUID CRYSTALS BOOK SERIES

DNA LIQUID-CRYSTALLINE DISPERSIONS AND NANOCONSTRUCTIONS



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Taylor & Francis Group

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Contents

Authors' Preface

Many of the results included in the book *DNA Liquid-Crystalline Dispersions and Nanoconstructions* were received at a time that was especially difficult for Russian science; they were achieved only because colleagues from various institutes of our country and other countries united in our endeavor in this area of science.

We have attempted to write this book in the hope that it will assist readers to see not only the many similarities between the properties of liquid-crystalline state of DNA and the peculiarities of the DNA state in living cells but also the very deep gap existing between properties of isolated DNA molecules in solution and the very complicated problems related to functioning DNA in cellular conditions.

We are greatly indebted to our coworkers and colleagues from the V. A. Engelhardt Institute of Molecular Biology of the Russian Academy of Sciences: Ya. M. Varshavsky, A. S. Tikhonenko, N. M. Akimenko, T. L. Pyatigorskaya, V. A. Kadykov, G. B. Lortkipanidze, A. L. Platonov, and N. S. Badaev, as well as to colleagues from other Russian institutes and universities: A. S. Sonin, V. P. Shibaev, L. M. Blinov, V. A. Belyakov, A. Yu. Grossberg, E. I. Kats, A. T. Dembo, V. G. Pogrebnyak, and V. P. Varlamov. We also acknowledge the assistance of colleagues from other countries: L. Lerman (US), B. Zimm (US), M. Maestre (US), C. Chandrasekhar (India), M. Palumbo (Italy), G. Gottarelli (Italy), F. Spener (Germany), and A. Turner (UK), who have stated various, sometimes very critical, but still well-wishing remarks when discussing certain steps of our investigations, and these have definitely contributed to the improvement of the quality of this book.

We are grateful to B. P. Gottikh (the Russian Academy of Sciences, Biological Department), whose knowledge and long-term scientific experience have contributed to more precise formulation of the general concept of this book.

We want to express our thanks to many students of various colleges and universities for the direct participation in the experiments described, in part, in our book.

For purely technical reasons, we cannot enumerate all of the participants at every stage of our studies, but their names and scientific contribution can be found in the references to the chapter of this book.

We are especially grateful to our wives, who, regardless of our (as well as all Russian scientists') complicated financial circumstances, created the conditions for our productive work related to the presentation of the scientific material included in this book.

Editor's Preface

When considering the known properties of one condensed state of polymeric molecules, namely, the liquid-crystalline state, and, especially, the lyotropic liquid-crystalline state of these molecules, we see a number of peculiarities that apparently have much in common with the properties of double-stranded DNA molecules in biological objects. First, some of the mechanisms of liquid crystals formation are realized almost without any expenditure of energy. Second, the x-ray parameters of the DNA molecules in artificially condensed phases are close enough to the parameters of the DNA molecules in biological objects. Third, there are many differing liquid-crystalline phases, and the transitions between them are regulated not only by the properties of DNA but also by the properties of the medium in which the condensed phases are obtained. Finally, not only the specific structure of liquid-crystalline phase itself but also that of the double-stranded DNA it contains is restored by almost 100% after the removal of the factors, such as temperature, inducing their "denaturation."

Double-stranded native DNA has two peculiarities that determine many of the physical properties of tightly packed DNA. Double-stranded DNA molecules have their own anisotropic properties because they contain both geometric anisometry (as the molecules are helical) and optical anisotropy (caused mainly by the presence of asymmetric carbon atoms in the structure of sugar residues). This is typical of the native DNA feature and cannot be changed in any way; it is an internal property that is inherent to native DNA only, under any condition. In the language of physics and physical chemistry of liquid crystals, this fact means that the fragments (molecules) of native DNA will always tend to so-called cholesteric packing as they approach one another. The double-stranded DNA molecules cannot be "deprived" of this property; they can only be affected by forcing DNA molecules or their complexes to pack into different liquid-crystalline phases. But at the first opportunity, the tendency of DNA molecules to form cholesterics will become apparent and dominate, determining the properties of the condensed phase of DNA.

A number of extra peculiarities of DNA liquid crystals are interesting from the biological point of view. On the one hand, a tailored modification of the DNA secondary structure in a liquid-crystal phase in a relatively broad extent does not result in the distortion of the mode of the spatial packing (i.e., the liquid-crystalline system has a high "structural memory" determined not by the properties of single molecules (fragments) of DNA but by the properties of the whole ensemble of neighboring molecules). On the other hand, at the "moment of formation" of a liquid crystal, when single fragments (molecules) of DNA and their complexes (for instance, with histone proteins) "recognize" one another, the properties of these fragments exert a significant, if not determining, influence on the mode of spatial packing, and relatively small changes in the structure of the single DNA molecule can regulate the spatial structure of the whole liquid-crystalline ensemble of the molecules.

This fact has allowed one of the authors of this book to illustrate a "gap" existing between the properties of DNA molecules freely floating in the laboratory solvent, where the linear responses to the external effects are observed, and the properties of the highly ordered liquid-crystalline state of these molecules, where DNAs have nonlinear properties and generate nonlinear responses to external effects. This means that the correct transfer of knowledge received when studying the properties of isolated DNA molecules compared to the properties of condensed DNA molecules in biological objects is not a simple problem, and must be solved in the near future. The authors illustrate, also, the existence of a very complicated problem related to the precise descriptions of functioning of DNA molecules under conditions of living cells. However, this book is not attempting an absolute definition of all these problems but rather is a general introduction to the subject.

In addition, the authors demonstrate that the experimental results obtained in studies of the fundamental problems can be used for resolving important and practical questions in medicine and biotechnology—for instance, how to obtain various types of biosensing units for bioanalytical systems.

It can be assumed that readers of this book specializing in various areas of the studies of living systems will find not only parts that can be criticized but also parts that could suggest many more possibilities of applying their own knowledge to explain the peculiarities of the packing and functioning of DNA molecules in biological objects. It can be expected that the input of physicists, chemists, and biologists dealing with the various aspects of living systems and interested, especially, in the biological processes that take place in living cells will bring to this field many new achievements that may be useful for molecular biology, nanobiotechnology, and applied medicine.*

Yu. M. Yevdokimov

* Note regarding this edition: Only minor changes were introduced in the text of the book and to the figures during translation of its contents from Russian to English.

Foreword

The discovery of the spatial structure of the double-stranded DNA molecule is one of the greatest achievements of science. It would not be an exaggeration to say that the DNA double helix is a distinguished symbol of modern biology, as its leading branches in the second half of the twentieth century were molecular biology and molecular genetics.

The deciphering of the replication mechanism of genetic information and the expression of the genome's basic vital processes have not only become the heritage of fundamental science but also determine the development of many areas of medical science, agriculture, and a number of industrial sectors.

DNA has been one of the most intensively studied objects for decades. The results of these researches are described in many thousands of scientific papers. Nevertheless, the simple-looking spatial "image" of complementary polynucleotide chains twisted into a helix still holds many secrets connected with the relationship between the structure and functions of DNA.

The January 2003 issue of *Nature* (Vol. 421), devoted to the fiftieth anniversary of the publication of an article by J. D. Watson and F. H. C. Crick entitled "Molecular structure of nucleic acids," contains a very interesting and remarkable statement by P. Ball: "The double helix is idealized for its aesthetic elegant structure, but the reality of DNA's physical existence is quite different. Most DNA in the cell is compressed into a tangled package that somehow still exposes itself to meticulous gene-regulatory control…. One has the impression of a genome as a book lying open, waiting to be read. However, it is not straightforward. The book is closed up, sealed, and packed away. Moreover, the full story is not merely what is written on the pages; these operations on DNA involve information transfer over many length scales.… We know about molecules; we know about cells and organelles; but the stuff in between is messy and mysterious."

This statement that the functioning of the "DNA package" cannot be understood is based on the iconic model of the Watson and Crick ideal double helix. This also shows that there is a gap in the "transfer" of the properties of isolated DNA molecules to the properties of packed (condensed) DNA form that really exists in a living cell. Moreover, the standard methods of bioinformatics used for the annotation of genomes depicted as linear DNA chains do not reflect the whole set of legitimacies (patterns) that determine the connection between a certain genome and the features of an individual person.

In this respect, the research and the modeling of both physicochemical properties and the biological activity of the condensed state of nucleic acids becomes especially important, considering the fact that this spatial form of DNA molecules can exist in the content of chromosomes.

The idea of collecting into a book the results that demonstrate the existence of a complicated relationship between the peculiarities of the condensed state of nucleic acid molecules and the functioning of these molecules in a cell was presented to

the editorial board office of *Technology of Living Systems* journal, since a group of authors had written a number of reviews concerning the issue and published them in this journal in 2007. The reviews have attracted the interest of a worldwide scientific audience.

The book *DNA Liquid-Crystalline Dispersions and Nanoconstructions* is an attempt to summarize the results received by scientists from different countries and laboratories that demonstrate the multiplicity and variability of condensed forms of nucleic acids. Here, in particular, the works of Russian scientists in the field of liquid-crystalline state of nucleic acids are well presented.

The conclusion that follows from the results represented in the book is that the phase exclusion of double-stranded linear or circular DNA molecules or their complexes with polycations induces transition of these molecules into a special liquid-crystalline state that is not at all biologically inert.

Moreover, the fundamental results so far have helped the authors to demonstrate the possible application of nucleic acid liquid crystals for practical purposes, namely, for the creation of nanoconstructions with unique physicochemical properties and sensing units for sensor devices used to detect chemical or biologically active compounds that affect the genetic material of a cell.

The hope is that the book will attract the attention of biologists, physicists, and chemists to this interesting area of science. It will be useful for both students and lecturers, and may contribute to the application of the concept of the condensed DNA for the study of various biological systems and their operational mechanisms.

A. I. Grigoriev

Introduction

Many years before physicists and chemists began to investigate liquid crystals of polymeric macromolecules, biologists had already supposed that some of biomacromolecules in living cells could adopt a specific structural condition that is now called the *liquid-crystalline state*.

The existence of a liquid-crystalline state of biological structures was first mentioned in 1933 at the Faraday Society Meeting, and, later in the 1970s, G. H. Brown and J. J. Wolken initiated a discussion to find out what the connection was between the distinctive properties of biological structures (and biological reactions) and the structure and properties of liquid crystals.

A number of original monographs and translated books were published meanwhile in Russian. The most remarkable of them are *The Physics and Chemistry of Life* (D. Flanagan, Moscow, Russian trans., 1960), *Coacervates and Protoplasm* (K. B. Serebrovskaya, Nauka-Edition, Moscow, 1971), *Periodical Colloid Structures* (I. F. Yefremov, Khimiya-Edition, Leningrad, 1971), *Liquid Crystal State of Polymers* (S. P. Papkov and V. G. Kulichikhin, Khimiya-Edition, Moscow, 1977), *The Physics of Liquid Crystals* (P. G. de Gennes Russian trans., Moscow, 1977), *Comb-Shaped Polymers and Liquid Crystals* (N. A. Plate, V. P. Shibaev, Khimiya-Edition, Moscow, 1980), *Liquid Crystals* (S. Chandrasekhar, Russian trans., Moscow, 1980), *Liquid Crystalline Order in Polymers* (A. Blumstein, Russian trans., Moscow, 1981), and *Liquid Crystals and Biological Structures* (G. H. Brown and J. J. Wolken, Russian trans., Moscow, 1982). The authors of these works touch on the properties of various liquid-crystalline biopolymers to some extent. However, they virtually left aside the issue of the liquid-crystalline state of nucleic acids.

This book was written as an attempt to consider the state of nucleic acid molecules in the cells and trace the possible connections between the peculiarities of this state and the functioning of nucleic acid molecules. We did not have the intention of covering all the issues of the nucleic acid molecules functioning under cell conditions; therefore, in Table I.1 we enumerate in chronological order experimental works of the authors who, in our opinion, made the most significant contribution to the formation of the current concept of the liquid-crystalline state of nucleic acid molecules and their peculiarities. Though this table does not cover all the works in this field, it shows that the works of Russian scientists are not inferior to any of the works of foreign authors, and many of them can be considered pioneers.

Further, there are references in every chapter to special literature that reflect the contributions of scientists from different countries to the solution of various aspects of problems related to the liquid-crystalline state of nucleic acids.

TABLE I.1
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
1.	1961	Robinson K.	Liquid-crystalline structures in polypeptide solutions	*Tetrahedron*, Vol. 13, pp. 219–234
2.	1971	Lerman L.S.	A transition to a compact form of DNA in polymer solutions	*Proc. Natl. Acad.* Sci. USA, Vol. 68, pp. 1886–1890
3.	1972	Evdokimov Yu.M., Platonov A.L., Tikhonenko A.S., Varshavsky Ya.M.	A compact form of double-stranded DNA in solution	*FEBS Lett.*, Vol. 23, pp. 180–184
4.	1973	Akimenko N.M., Dijakova E.B., Evdokimov Yu.M., Frisman E.V., Varshavsky Ya.M.	Viscosimetric study on compact form of DNA in water-salt solutions containing polyethyleneglycol	*FEBS Lett.,* Vol. 38, pp. 61–63
5.	1973	Evdokimov Yu.M., Akimenko N.M., Glukhova N.E., Tikhonenko A.S., Varshavsky Ya.M.	Formation of the compact form of double-stranded DNA in solution in the presence of polyethylene glycol	*Molecular Biology* (Russian edition), Vol. 7, pp. 151–159
6.	1974	Maniatis T., Venable J.H., Lerman L.S.	The structure of ψ DNA	*J. Mol. Biol.,* Vol. 84, pp. 37–64
7.	1976	Gosule L.C., Schellman J.A.	Compact form of DNA induced by spermidine	*Nature*, Vol. 259, pp. 333–335
8.	1977	Evdokimov Yu.M., Pyatigorskaya T.L., Kadikov V.A., Polyvtsev O.F., Doscočil J., Koudelka Ya., Varshavsky Ya.M.	DNA compact form in solution: 12. A formation of a compact form of double-stranded RNA in the presence of poly(ethylene glycol)	*Molecular Biology* (Russian edition), Vol. 11, pp. 891–900
9.	1977	Iizuka I.	Some new findings in the liquid crystals of sodium salt of desoxyribonucleic acid	*Polymer J.,* Vol. 9, pp. 173–180
10.	1977	Sipski M.L., Wagner T.E.	Probing DNA quaternary ordering with circular dichroism spectroscopy studies of equine sperm chromosomal fibers	*Biopolymers*, Vol. 16, pp. 573–582

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
11.	1978	Skuridin S.G., Kadykov V.A., Shashkov V.S., Evdokimov Yu.M., Varshavsky Ya.M.	The formation of a compact form of DNA in solution induced by interaction with spermidine	*Molecular Biology* (Russian edition), Vol. 12, pp. 413–420
12.	1979	Wilson R.W., Bloomfield V.A.	Counterion-induced condensation of deoxyribonucleic acid	*Biochemistry*, Vol. 18, pp. 2192–2196
13.	1979	Becker M., Misselwitz R., Damaschun H., Damaschun G., Zirver D.	Spermine–DNA complexes build up metastable structures: small-angle X-ray scattering and circular dichroism studies	*Nucl. Acids Res*., Vol. 7, pp. 1297–1309
14.	1980	Widom J., Baldwin R.L.	Cation-induced toroidal condensation of DNA: studies with $Co^{3+}(NH_3)_6$	*J. Mol. Biol.,* Vol. 144, pp. 431–453
15.	1980	Tinoko I., Bustamante C., Maestre M.F.	The optical activity of nucleic acids and their aggregates	*Annu. Rev. Biophys. Bioeng*., Vol. 9, pp. 107–146
16.	1981	Potaman V.N., Alexeev D.G., Skuratovskii I.Ya., Rabinovich A.Z., Shlyakhtenko L.S.	Study of DNA films by the CD, X-ray and polarization microscopy techniques	*Nucl. Acids. Res.,* Vol. 9, pp. 55–64
17.	1981	Huey R., Mohr S.C.	Condensed state of nucleic acids: III. $\psi_{(+)}$ and $\psi_{(-)}$ conformational transitions of DNA induced by ethanol and salt	*Biopolymers*, Vol. 20, pp. 2533–2552
18.	1981	Allison S.A., Herr J.C., Schurr J.M.	Structure of viral φ29 DNA condensed by simple triamines: light-scattering and electron microscopy study	*Biopolymers*, Vol. 20, pp. 469–488
19.	1983	Rill R.L., Hilliard P.R., Levy G.C.	Spontaneous ordering of DNA	*J. Biol. Chem.,* Vol. 258, pp. 250–256

continued

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
20.	1984	Livolant F.	Cholesteric organization of DNA *in vivo* and *in vitro*	*Eur. J. Cell Biol.,* Vol. 33, pp. 300–311
21.	1984	Marx K.A., Ruben G.C.	Studies of DNA organization in hydrated spermidine-condensed DNA toruses and spermidine-DNA fibres	*J. Biomol. Struct. Dynam*., Vol. 1, pp. 1109–1132
22.	1986	Brandes R., Kearns D.R.	Magnetic ordering of DNA liquid crystals	*Biochemistry*, Vol. 25, pp. 5890–5895
23.	1986	Rill R.L.	Liquid crystalline phases in concentrated aqueous solutions of Na^+ DNA	*Proc. Natl. Acad. Sci. USA*, Vol. 83, pp. 342–346
24.	1986	Livolant F.	Cholesteric liquid crystalline phases given by three helical biological polymers: DNA, PBLG and xanthan: a comparative analysis of their textures	*J. Physique*, Vol. 47, pp. 1605–1616
25.	1986	Livolant F., Bouligand Y.	Liquid crystalline phases given by helical biological polymers (DNA, PBLG and xanthan) Columnar textures	*J. Physique*, Vol. 47, pp. 1813–1827
26.	1987	Livolant F.	Precholesteric liquid crystalline states of DNA	*J. Physique*, Vol. 48, pp. 1051–1066
27.	1987	Strzelecka T.E., Rill R.L.	Solid-state ^{31}P NMR studies of DNA liquid crystalline phases: the isotropic to cholesteric transition	*J. Amer. Chem.* Soc., Vol. 109, pp. 4513–4518
28.	1987	Baeza I., Gariglio P., Rangel L.M., Chavez P., Cervantes L., Arguello C.	Electron microscopy and biochemical properties of polyamine-compacted DNA	*Biochemistry,* Vol. 26, pp. 6387–6392

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
29.	1988	Yevdokimov Yu.M., Skuridin S.G., Salyanov V.I.	The liquid-crystalline phases of double-stranded nucleic acids *in vitro* and *in vivo*	*Liq. Crystals*, Vol. 3, pp. 1443–1459
30.	1988	Livolant F., Maestre M.F.	Circular dichroism microscopy of compact forms of DNA and chromatin *in vivo* and *in vitro*: cholesteric liquid-crystalline phases of DNA and single dinoflagellate nuclei	*Biochemistry*, Vol. 27, pp. 3056–3068
31.	1988	Strzelecka T.E., Davidson M.W., Rill R.L.	Multiple liquid crystal phases of DNA at high concentrations	*Nature*, Vol. 331, pp. 457–460
32.	1988	Spada G.P., Brigidi P., Gottarelli G.	The determination of the handedness of cholesteric superhelices formed by DNA fragments	*J. Chem. Soc., Chem. Commun.*, Vol. 14, pp. 953–954
33.	1989	Rill R.L., Livolant F., Aldrich H.C., Davidson M.W.	Electron microscopy of liquid crystalline DNA: direct evidence for cholesteric-like organization of DNA in dinoflagellate chromosomes	*Chromosoma* (Berl.), Vol. 98, pp. 280–286
34.	1989	Livolant F.	Lines in liquid crystalline phases of biopolymers	*J. Phys. France*, Vol. 50, pp. 1729–1741
35.	1989	Livolant F., Levelut A.M., Doucet J., Benoit J.P.	The highly concentrated liquid-crystalline phase of DNA is columnar hexagonal	*Nature*, Vol. 339, pp. 724–726
36.	1989	Torbet J., DiCapua E.	Supercoiled DNA is interwound in liquid crystalline solutions	*EMBO J.,* Vol. 8, pp. 4351–4356
37.	1990	Van Winkle D.H., Davidson M.W., Wan-Xu Chen, Rill R.L.	Cholesteric helical pitch of near persistence length DNA	*Macromolecules*, Vol. 23, pp. 4140–4148

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
38.	1990	Strzelecka T.E., Rill R.L.	Phase transitions of concentrated DNA solutions in low concentrations of 1:1 supporting electrolyte	*Biopolymers*, Vol. 30, pp. 57–71
39.	1991	Salyanov V.I., Dembo A.T., Yevdokimov Yu.M.	Liquid-crystalline phases of circular superhelical DNA and their modification by the action of nuclease enzymes	*Liq. Crystals*, Vol. 9, pp. 229–238
40.	1991	Livolant F.	Supramolecular organization of double-stranded DNA molecules in the columnar hexagonal liquid crystalline phase: an electron microscopic analysis using freeze-fracture methods	*J. Mol. Biol*., Vol. 218, pp. 165–181
41.	1991	Rill R.L., Strzelecka T.E., Davidson M.W., van Winkle D.H.	Ordered phases in concentrated DNA solutions	*Physica*, Vol. A 176, pp. 87–116
42.	1991	Livolant F.	Ordered phases of DNA *in vitro* and *in vivo*	*Physica*, Vol. A 176, pp. 117–137
43.	1991	Bloomfield V.	Condensation of DNA by multivalent cations: considerations on mechanism	*Biopolymers*, Vol. 31, pp. 1471–1481
44.	1991	Gottarelli G., Spada G.P., Miranda de Morais M.	The effect of ethidium bromide on the liquid crystalline phases of aqueous DNA	*Chirality*, Vol. 3, pp. 227–232
45.	1991	Baeza I., Ibanez M., Wong C., Chavez P., Gariglio P., Oro J.	Possible prebiotic significance of polyamines in the condensation, protection, encapsulation, and biological properties of DNA	*Orig. Life Evol. Biosph.,* Vol. 21, pp. 225–242
46.	1992	Yevdokimov Yu.M., Skuridin S.G., Lortkipanidze G.B.	Liquid-crystalline dispersions of nucleic acids	*Liq. Crystals*, Vol. 12, pp. 1–16

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
47.	1992	Ghirlando R., Wachtel E.J., Arad T., Minsky A.	DNA packaging induced by micellar aggregates: a novel *in vitro* DNA condensation system	*Biochemistry*, Vol. 31, pp. 7110–7119
48.	1992	Rau D.C., Parsegian V.A.	Direct measurement of the intermolecular forces between counterion-condensed DNA double helices	*Biophys. J.,* Vol. 61, pp. 246–259
49.	1992	Durand D., Doucet J., Livolant F.	A study of the structure of highly concentrated phases of DNA by x-ray diffraction	*J. Phys.* (France), Vol. 2, pp. 1769–1783
50.	1993	Leforestier A., Livolant F.	Supramolecular ordering of DNA in the cholesteric liquid crystalline phase: an ultrastructural study	*Biophys. J.,* Vol. 65, pp. 56–72
51.	1994	Leforestier A., Livolant F.	DNA liquid crystalline blue phases: electron microscopy evidence and biological implications	*Liq. Crystals*, Vol. 17, pp. 651–658
52.	1994	Merchant K., Rill R.L.	Isotropic to anisotropic phase transition of extremely long DNA in an aqueous saline solution	*Macromolecules*, Vol. 27, pp. 2365–2370
53.	1994	Gottarelli G., Spada G.P.	Application of CD to the study of some cholesteric mesophases (In: *Circular Dichroism: Principles and Applications*)	New York: VCH, pp. 105–119
54.	1994	Robinov C., Kellenberger E.	The bacterial nucleoid revisited	*Microbiol. Rev.,* Vol. 58, pp. 211–232
55.	1994	Sikorav J.-L., Pelta J., Livolant F.	A liquid crystalline phase in spermidine-condensed DNA	*Biophys. J.*, Vol. 67, pp. 1387–1392

continued

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
56.	1994	Reich Z., Wachtel E.J., Minsky A.	Liquid-crystalline mesophases of plasmid DNA in bacteria	*Science,* Vol. 264, pp. 1460–1463
57.	1994	Reich Z., Levin-Zaidman S., Gutman S.B., Arad T., Minsky A.	Supercoiling-regulated liquid-crystalline packaging of topologically constrained, nucleosome-free DNA molecules	*Biochemistry*, Vol. 33, pp. 14177–14184
58.	1996	Yevdokimov Yu.M., Salyanov V.I., Gedig E., Spener F.	Formation of polymeric chelate bridges between double-stranded DNA molecules fixed in spatial structure of liquid-crystalline dispersions	*FEBS Lett.,* Vol. 392, pp. 269–273
59.	1996	Livolant F., Leforestier A.	Condensed phases of DNA: structures and phase transitions	*Prog. Polym. Sci*., Vol. 21, pp. 1115–1164
60.	1996	Pelta J., Livolant F., Sikorav J.-L.	DNA aggregation induced by polyamines and cobalthexamine	*J. Biol. Chem*., Vol. 271, pp. 5656–5662
61.	1996	Pelta J., Durand D., Doucet J., Livolant F.	DNA mesophases induced by spermidine: structural properties and biological implications	*Biophys. J.,* Vol. 71, pp. 48–63
62.	1996	Leforestier A., Richter K., Livolant F., Dubochet J.	Comparison of slam-freezing and high-pressure freezing effects on the DNA cholesteric liquid crystalline structure	*J. Microsc.,* Vol. 184 (Pt. 1), pp. 4–13
63.	1997	Leforestier A., Livolant F.	Liquid crystalline ordering of nucleosome core particles under macromolecular crowding conditions: evidence for a discotic columnar hexagonal phase	*Biophys. J.,* Vol. 73, pp. 1771–1776

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
64.	1998	Kassapidou K., Jesse W., van Dijk J.F., van der Maarel J.R.	Liquid crystal formation in DNA fragment solutions	*Biopolymers*, Vol. 46, pp. 31–37
65.	1998	Yevdokimov Yu.M., Salyanov V.I., Lortkipanidze G.B., Gedig E., Spener F., Palumbo M.	Sensing biological effectors through the response of bridged nucleic acids and polynucleotides fixed in liquid-crystalline dispersions	*Biosens. Bioelectron.*, Vol. 13, pp. 279–291
66.	1998	Lin Z., Wang C., Feng X., Liu M., Li J., Bai C.	The observation of the local ordering characteristics of spermidine-condensed DNA: atomic force microscopy and polarization microscopy studies	*Nucl. Acids Res.,* Vol. 26, pp. 3228–3234
67.	1999	Saminathan M., Antony M., Shirahata A., Sigal L.H., Thomas T., Thomas T.J.	Ionic and structural specificity effects of natural and synthetic polyamines on the aggragation and resolubilization of single-, double-, and triple-stranded DNA	*Biochemistry,* Vol. 38, pp. 3821–3830
68.	1999	Wolf S.G., Frenkiel D., Arad T., Finkel S.E., Kolter R., Minsky A.	DNA protection by stress-induced biocrystallization	*Nature,* Vol. 400, pp. 83–85
69.	2000	Yevdokimov Yu.M., Salyanov V.I., Skuridin S.G., Dembo A.T., Platonov Yu.V., Il·ina A.V., Varlamov V.P.	Complexes of the (dsDNA–Chitosan) form cholesteric liquid-crystalline dispersions	*Doklady Academii Nauk* (formerly *Doklady of the USSR Academy of Sciences*), Vol. 374, pp. 696–698 (Russian edition)
70.	2001	Yevdokimov Yu.M., Salyanov V.I., Zakharov M.A.	A novel type of microscopic size chip based on double-stranded nucleic acids	*Lab on a Chip*, Vol. 1, pp. 35–41

continued

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
71.	2001	Vijayanathan V., Thomas T., Shirahata A., Thomas T.J.	DNA condensation by polyamines: a laser light scattering study of structural effects	*Biochemistry*, Vol. 40, pp. 13644–13651
72.	2001	Bouligand Y., Norris V.	Chromosome separation and segregation in dinoflagellates and bacteria may depend on liquid crystalline states	*Biochimie*, Vol. 83, pp. 187–192
73.	2001	Sartori B.N., Senn A., Leforestier A., Livolant F., Dubochet J.	DNA in human and stallion spermatozoa forms local hexagonal packing with twist and many defects	*J. Struct. Biol.*, Vol. 134, pp. 76–81
74.	2001	Piraccini S., Gottarelli G., Mariani P., Spada G.P.	The chirality of the cholesteric phases of DNA and G-wires: its connection to their molecular structures	*Chirality*, Vol. 6, pp. 3249–3253
75.	2002	Saminathan M., Thomas T., Shirahata A., Pillai C.K., Thomas T.J.	Polyamine structural effects on the induction and stabilization of liquid crystalline DNA: potential applications to DNA packaging, gene therapy and polyamine therapeutics	*Nucl. Acids Res*., Vol. 30, pp. 3722–3731
76.	2002	Kato T.	Self-assembly of phase-segregated liquid crystal structures	*Science,* Vol. 295, pp. 2414–2418
77.	2002	Zakharova S.S., Jesse W., Backendorf C., van der Maarel J.R.	Liquid crystal formation in supercoiled DNA solutions	*Biophys. J.,* Vol. 83, pp. 1119–1129
78.	2002	Goobes R., Cohen O., Minsky A.	Unique condensation patterns of triplex DNA: physical aspects and physiological implications	*Nucl. Acids Res*., Vol. 30, pp. 2154–2161

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
79.	2004	Vijayanathan V., Thomas T., Antony M., Shirahata A., Thomas T.J.	Formation of DNA nanoparticles in the presence of novel polyamine analogues: a laser light scattering and atomic force microscopic study	*Nucl. Acids Res*., Vol. 32, pp. 127–134
80.	2004	Englander J., Klein E., Brumfeld V., Sharma A.K., Doherty A.J., Minsky A.	DNA toroids: framework for DNA repair in Deinococcus radiodurans and in germinating bacterial spores	*J. Bacteriol.,* Vol. 186, pp. 5973–5977
81.	2004	Gottarelli G., Spada G.P.	The stepwise supramolecular organization of guanosine derivatives	*Chem. Rec.,* Vol. 4, pp. 39–49
82.	2005	Yevdokimov Yu.M., Skuridin S.G., Nechipurenko Yu.D., Zakharov M.A., Salyanov V.I., Kurnosov A.A., Kuznetsov V.D., Nikifirov V.N.	Nanoconstructions based on double-stranded nucleic acids	*Int. J. Biol. Macromol.,* Vol. 36, pp. 103–115
83.	2005	Yevdokimov Yu.M., Salyanov V.I., Kondrashina O.V., Borshevsky V.I., Semenov S.V., Gasanov A.A., Reshetov I.V., Kuznetsov V.D., Nikiforov V.N., Akulinichev S.V., Mordovskoi M.V., Potashev S.I., Skorkin V.M.	Particles of liquid-crystalline dispersions formed by (nucleic acid–rare earth element) complexes as a potential platform for neutron capture therapy	*Int. J. Biol. Macromol.,* Vol. 37, pp. 165–173

continued

TABLE I.1 (continued)
Chronology of the Most Significant Works Devoted to the Experimental Research of Peculiarities of Nucleic Acids' Condensed State

Order of Publication	Year	Author(s)	Paper Title	Journal
84.	2005	Wong J.T., Kwok A.C.	Proliferation of dinoflagellates: blooming or bleaching	*Bioassays,* Vol. 27, pp. 730–740
85.	2006	Sundaresan N., Thomas T., Thomas T.J., Pillai C.K.	Lithium ion induced stabilization of the liquid crystalline DNA	*Macromol. Biosci.,* Vol. 6, pp. 27–32
86.	2006	Smalyukh I.I., Zribi O.V., Butler J.C., Lavrentovich O.D., Wong G.C.	Structure and dynamics of liquid crystalline pattern formation in drying droplets of DNA	*Phys. Rev. Lett.,* Vol. 96, pp. 177801
87.	2006	Safinya C.R., Ewert K., Ahmand A., Evans H.V., Rativ U., Needleman D.J., Lin A.J., Slack N.L., George C., Samuel C.	Cationic liposome-DNA complexes: from liquid crystal science to gene delivery applications	*Philos. Transact. A Math. Phys. Eng. Sci.,* Vol. 364, pp. 2573–2596
88.	2006	Livolant F., Mangenot S., Leforestier A., Dertin A., Frutos M., Raspaud E., Durand D.	Are liquid crystalline properties of nucleosomes involved in chromosome structure and dynamics?	*Philos. Transact. A Math. Phys. Eng. Sci.,* Vol. 364, pp. 2615–2633

PECULIARITIES OF DNA MOLECULES IN SOME BIOLOGICAL OBJECTS

The results obtained in different laboratories up to the present time allow us to enumerate the distinctive properties of the DNA molecules and their complexes with some proteins inside biological objects.

To estimate the amount of DNA required to provide the functioning of different biological objects, let us project the length of their DNAs. According to evaluations [1], the amount of DNA necessary to describe an organism (its genome) is enormous. For instance, even the DNA of a simple bacterium contains 10^6 nucleotide pairs. Eukaryotes, such as mammals and plants, have about 10^{10} nucleotide pairs. A human genome contains $4{\cdot}10^9$ nucleotide pairs. This genome is represented by two copies in each somatic cell so that each cell contains $8{\cdot}10^9$ nucleotide pairs. If all of this DNA were to be laid out in a line, considering that the length of one pair of bases in a B-form of the DNA is 0.34 nm, it would extend about 2.7 m. Given that there are around 10^{13} cells in a human body, there are $3{\cdot}10^{10}$ km of DNA in any person. However, about 90% of this DNA is latent (silent?), and its functions are still uncertain. DNA is spread around every cell in the form of chromosomes. A human cell

has 46 chromosomes. Each of them contains approximately $1.7 \cdot 10^8$ nucleotide pairs and is about 6 cm long.

In the smallest human chromosome, the length of a DNA 14 mm long is condensed into a chromosome about 2 μm, with a packing ratio of 7000. Hence, the packing ratio for DNA is enormous.

Therefore, the first question of interest is how to evaluate the local DNA concentration as weight of the DNA per a unit of cell volume for different biological objects (which is also called the *packing density*).

In general, the estimation of the packing density of DNA in different biological objects is nontrivial. In exponentially growing cells of *E. coli*, the DNA concentration reaches 4% of the cell solid material, which is approximately $15 \cdot 10^{-15}$ g [2]. If the DNA is homogeneously dispersed in the cell with a volume of $1.4 \cdot 10^{-12}$ cm^3, the packing density of the DNA must be about 10 mg/mL. On average, there are less than 3 genomes in an *E. coli* cell, which conforms to cytological findings (2–4 genomes per cell). Considering DNA concentration in nucleotides and the fact that a volume of a nucleotide is 30–70% of a cell's volume, we can obtain a quantity of 20 to 50 mg/mL.

For the interphase nuclei of hepatocytes, an average DNA concentration is 20–40 mg/mL, considering that there is $6–12 \cdot 10^{-12}$ g of DNA in each cell, and the nucleus volume is $2.8 \cdot 10^{-10}$ cm^3. The possible difference of DNA concentrations in heterochromatin and euchromatin is set aside. It is also interesting that the DNA concentration in *E. coli* is comparable with concentration typical of the hepatocytes' interphase nuclei.

DNA can be packed even more densely. For some objects such as bacteriophages, the packing density can be calculated quite accurately. The calculations of the DNA packing density of bacterial viruses are based on the comparison of the bacteriophages' head volume and chemically detected DNA concentration in these objects [3]. The results of such calculations vary from 800 mg/mL for the T4 bacteriophage (calculation for this bacteriophage is the most accurate) to 350 mg/mL for the T3 bacteriophage. Calculation of the local DNA concentration in metaphase chromosomes is much more complicated, and there is hope that it can be estimated by atomic force microscopy.

Some of the data [4] on DNA packing density are given in Table I.2.

That the DNA local packing density in typical eukaryotic cells of animals and plants constantly changes because the diffuse chromatin "condenses" to the

TABLE I.2
DNA Packing Density in Various Objects

	Packing Density, mg/mL	
Object	**Calculated**	**Measured**
Bacteriophage T4 head	800	1000–2000
E. coli nucleoid	20–50	100 ± 50
Dinoflagellate chromosome	—	220 ± 80
CV-1 cell metaphase chromosome	—	300–500