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Liquid Crystalline Phases of DNA

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Abstract

The DNA molecule is the support of the genetic information. It is a right-handed double helix, 22 angstrom in diameter, and 50 nm in the persistence length. The two strands of the helix are complementary in their nucleotide sequence with about 10 nucleotide pairs per helical turn. DNA is a long and strongly charged heteropolymer. It bears on average one elementary negative charge per each 0.17 nm of the double helix. In the late 1940s, the ability of duplex DNA to form liquid crystal (LC) phases when hydrated was known. Since that time, the LC phases of solutions of duplex B-form DNA (B-DNA) have been extensively characterized. The chain length N of DNA ranges from mega-base pair (bp) semiflexible polymers down to approximately 100 bp rigid rodlike segments. These studies of long DNA (lDNA) have revealed cholesteric, columnar hexagonal and blue liquid crystalline phases. In 2007, Michi Nakata and his collaborators found nematic and columnar liquid crystal phases in the short complementary B-form DNA oligomers with 6 to 20 base pairs in length. Structural study shows that these phases are produced by the end-to-end adhesion and consequent stacking of the duplex oligomers into polydisperse anisotropic rod-shaped aggregates, which order into liquid crystals.

Contents

1 DNA molecule

1.1 Introduction [1][2]

DNA was first isolated by the Swiss physician Friedrich Miescher who, in 1869, discovered a microscopic substance in the pus of discarded surgical bandages. As it resided in the nuclei of cells, he called it "nuclein". For a long time the connection between nucleic acid and genes was not known.
In 1919, Phoebus Levine identified the base, sugar and phosphate nucleotide units.
In 1928, Frederik Griffith suggested that DNA could carry the genetic information.
In 1937, Willian Astbury produced the first X-ray diffraction patterns that showed that DNA had a regular structure.
In 1943, Oswald Avery, along with coworkers Colin Macleod and Maclyn Mccarty identified DNA as the transforming principle.
In 1952, Alfred Hershey confirmed the DNA's role in heredity.
In 1952, Rosalind Franklin and Raymond Gosling took an X-ray diffraction image of DNA.
In 1953 James Watson and Francis Crick presented the structure of the DNA-helix, the molecule that carries genetic information from one generation to the other. Nine years later, in 1962, they shared the Nobel Prize in Physiology or Medicine with Maurice Wilkins, for solving one of the most important of all biological riddles.

1.2 Structure of DNA

The structure of DNA is illustrated by a right handed double helix, with 3.4 nm length in one helical turn and about 10 nucleotide pairs per helical turn. Two polynucleotide chains, held together by weak thermodynamic forces, form a DNA molecule (figure 1). This structure was first described by James Watson and Francis Crick in 1953.

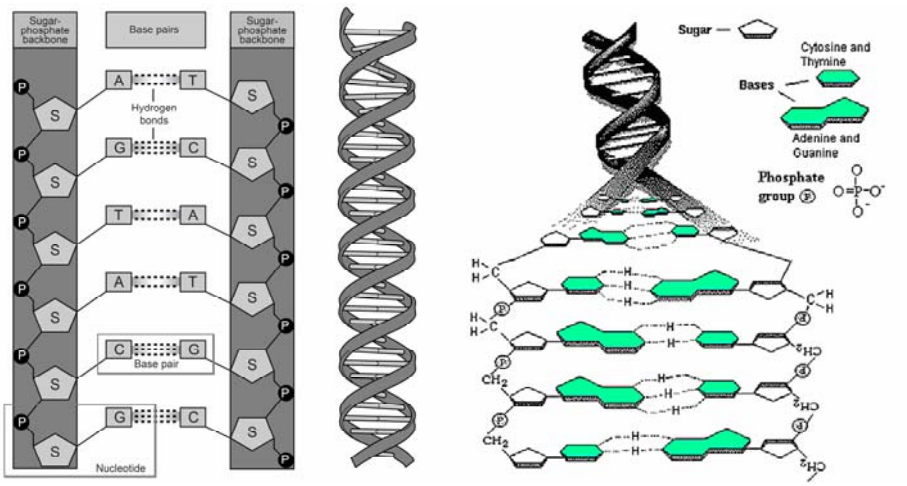


Figure 1. The double helix of the DNA is shown along with details of how the bases, sugars and phosphates connect to form the structure of the molecule.

1.2.1 Components of DNA [3][4]

DNA is a polymer. The monomer units of DNA are nucleotides, and the polymer is known as a "polynucleotide". Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogen containing base attached to the sugar, and a phosphate group (figure 1). There are four different types of nucleotides found in DNA, differing only in the nitrogenous base. The four nucleotides are given one letter abbreviations as shorthand for the four bases.

- A is for adenine
- G is for guanine
- C is for cytosine
- T is for thymine

A and T are connected by two hydrogen bonds. G and C are connected by three hydrogen bonds.

Adenine and guanine are purines. Purines are the larger of the two types of bases found in DNA. Structures are shown below:

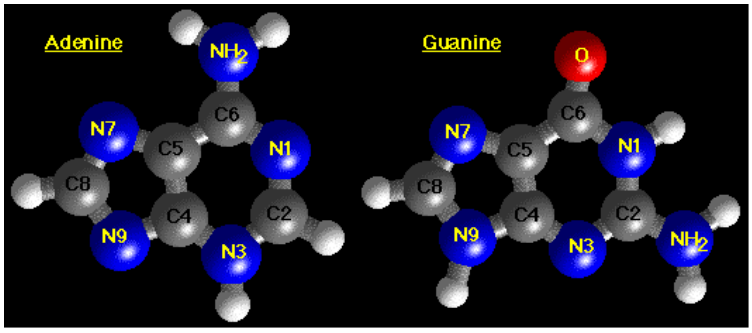


Figure 2. Structure of A and G. The 9 atoms that make up the fused rings (5 carbon, 4 nitrogen) are numbered 1-9. All ring atoms lie in the same plane.

Cytosine and thymine are pyrimidines. The 6 atoms (4 carbon, 2 nitrogen) are numbered 1-6. Like purines, all pyrimidine ring atoms lie in the same plane. (figure 3)

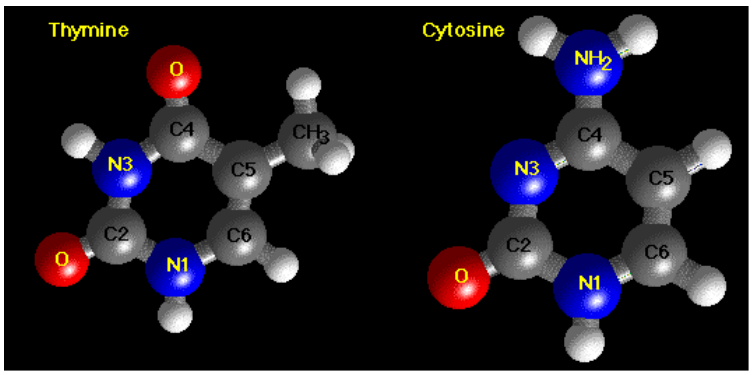


Figure 3. Structure of C and T.

1.2.2 Features of the DNA Double Helix [4]

DNA is a normally double stranded macromolecule. Two polynucleotide chains, held together by weak thermodynamic forces, form a DNA molecule.

- Two DNA strands form a helical spiral, winding around a helix axis in a right-handed spiral
- The two polynucleotide chains run in opposite directions
- The sugar-phosphate backbones of the two DNA strands wind around the helix axis like the railing of a sprial staircase
- The bases of the individual nucleotides are on the inside of the helix, stacked on top of each other like the steps of a spiral staircase.

2 Liquid crystalline phases of DNA

2.1 Liquid crystal phases [5]

Liquid crystals (LCs) are a state of matter that has properties between those of a conventional liquid and those of a solid crystal. LC may flow like a liquid, but its molecules may be oriented in a crystal-like way. There are many different types of LC phases. The various LC phases (called mesophases) can be characterized by the type of ordering. Positional order is that molecules are arranged in any sort of ordered lattice, and orientational order is pointing in the

same direction. According to the dimensionality of the translational correlations of building units, there are four basic types of liquid crystalline phases: nematic (no translational correlations), smectic (1D correlations), columnar (2D correlations), and various 3D-correlated structures, such as cubic phases. Liquid crystals are made of strongly anisometric molecules, either elongated (calamitic molecules) or disk-like (discotic molecules). As a rule, the inner part of mesogenic molecules is rigid (phenyl groups) and the outer part flexible (aliphatic chains). This double character explains altogether the existence of steric interactions (between rod-like or disk-like cores of the molecules) yielding orientational order and the fluidity of the mesomorphic phases.

2.2 Liquid crystal phases of long DNA [6][7]

The ability of duplex DNA to form liquid crystals (LC) phases was found in the late 1940s. Since that time, the LC phases of solution of duplex B-form DNA (B-DNA) have been extensively characterized optical, X-ray, and magnetic resonance methods. These studies have revealed an isotropic phase (I), chiral nematic (N*), blue and hexagonal liquid crystal phases with increasing DNA concentration (figure 4).

Linear DNA fragments in aqueous solution form multiple liquid crystal phases whose nature depends on the polymer concentration. By polarizing and electron microscopy and both methods, even X-ray diffraction method, when increasing the polymer concentration, the phases sequence is described quite precisely, as schematically presented here:

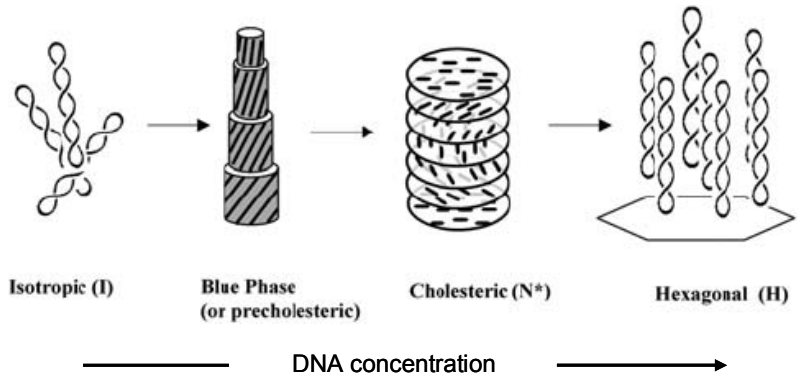
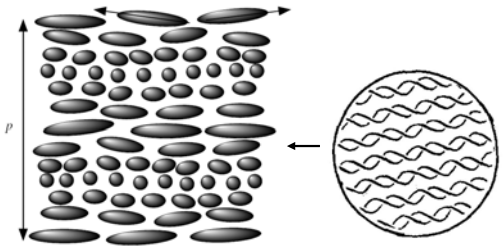


Figure 4. When increasing the DNA concentration, the isotropic slution transforms into either blue phase or precholesteric stage and then into a cholesteric phase which turns itself into columnar hexagonal. More concentrated phase is true crystal. Table 1 is the survey of the main experimental data with the indication of the DNA sources and ionic environment (in most cases K^+ Na^+ and NH_4^+ at concentrations ranging from about 10 mM to 3 M) .

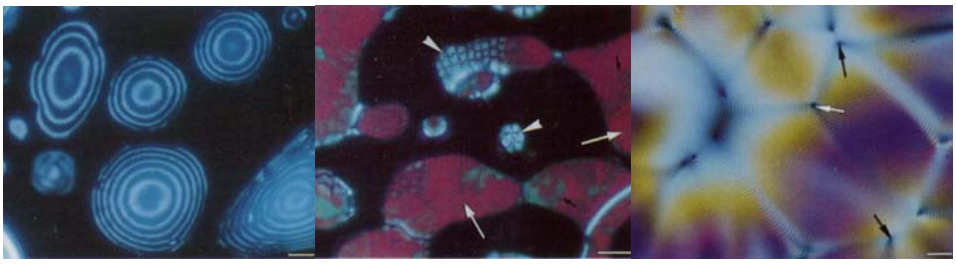
2.2.1 Cholesteric phase

Cholesteric phase is sometimes called chiral nematic phase. In the cholesteric phase, molecules are aligned in parallel and their orientation rotates continuously along a direction that is called the cholesteric axis. The twist of the molecules is perpendicular to the director (figure 5). Though this structure is continuous, for clarity we can draw a series of parallel and equidistant planes. Figure 6 shows the textures of cholestric phase of DNA.



a b

Figure 5. Schematic representation of the cholesteric organization. Double stranded DNA helices are aligned in parallel (b) and their orientation rotates along the cholestric axis p (a).



a b c

Figure 6. a, planar textures of the cholesteric phase of small droplets
b, planar textures (arrows) and polygonal textures (arrowheads) for the cholesteric phase
c, classical fingerprint patterns of the cholesteric phase. (white arrows: $-\pi$ disclinations, black arrows: $+\pi$ disclinations)

2.2.2 Blue phase

The choelsteric liquid crystals are the sequence of 'smectic-helical-isotropic' in the traditional phase diagram, as the temperature increases. Four blue phases are observed in a very narrow (approximately 1 degrees) temperature range between the isotropic and helical phase of cholesteric liquid crystal. They are BPI, BPII, BPIII and smectic blue phases. BPI and BPII both have long-range orientational order which has a 3D cubic symmetry, while the BPIII is isotropic and is only present in very chiral compounds. The smectic blue phase shows layer ordering. At least two of them are reported in the DNA lyotropic system. One corresponds to a loose random network of double twist cylinders floating

within an isotropic liquid; the other is a dense network of double twist bundles that fill the whole space.

Blue phases occur because the helicoidal structure of the chiral nematic phase is not the lowest energy configuration for chiral molecules. In the chiral nematic, molecules lie in quasi-nematic layers and rotate when going from one layer to the next. However, the free energy is actually lower if the molecules twist in two dimensions simultaneously. This leads to the formation of the double twist cylinder, where the molecules all rotate about a central axis.

The double twist cylinder is not stable over large distances (this is known as 'frustration', and hence blue phases are often termed 'frustrated phases'). The twist of a cylinder is limited to a maximum angle of 45 degrees between the directors of the axial molecules and those at the outer surface of the cylinder (figure 7). The cylinders therefore pack together to form cubic structures, interlaced with disclinations. For example, BPII has been shown to have a simple cubic structure, whilst BPI packs into a face-centred cubic (fcc) structure (figure 8).

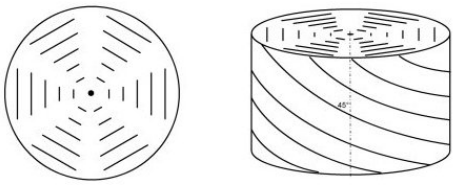


Figure 7.The double twist cylinder structure from above (left) and from a perspective view (centre).

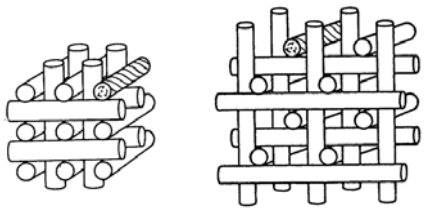
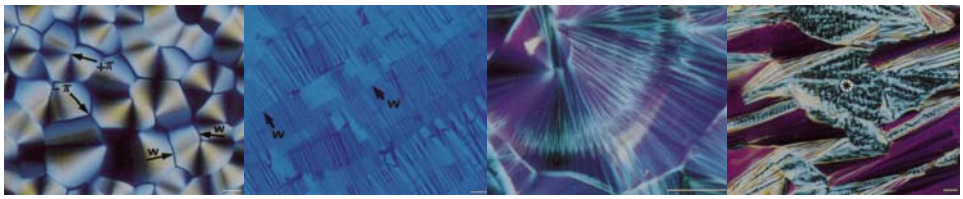


Figure 8. Cylinders stack in a variety of (usually mutually orthogonal) configurations to form the different blue phases. In BPI, the cylinders pack into a face-centred cubic structure (right), and in BP II, the cylinders pack into a simple cubic structure (left).

2.2.3 Columnar hexagonal phase

Columnar phases show 2D long-range positional order with translational

symmetries. In this phase, the molecules are unidirectionally aligned with a lateral hexagonal order. The true crystal is: molecules present some disorder around their position in the hexagonal array and the columns of the molecules generally show a parallel bend and are able to slide with respect to each other. Each molecule is free to rotate around its longitudinal axis. Figure 9 shows the textures of columnar hexagonal phase of DNA



a b c d

Figure 9. a, moderately concentrated columnar hexagonal phase. The texture are supple and show numerous $+\pi$ and $-\pi$ declinations. The walls (w) separate domains of different molecular orientation. b, undulating texture of the columnar hexagonal phase. Large bands are separated by walls (w) on both parts of which undulations are out of phase. c, columnar hexagonal phase with striated domains revealing a high DNA concentration. d, highly concentrated columnar hexagonal phase. The textures appear as a mosaic of small domains (*).

2.3 Liquid crystal phases of short DNA [8]

As mentioned above, there are various liquid crystal phases in the long DNA with chain length N ranging from mega-base pair (bp) semiflexible polymers down to approximately 100 bp rigid rodlike segments, comparable in size to the B-DNA persistence length (about 50 nm). For a long time, it has been confirmed that there should be no LC phase for L/D< 4.7 (N<28). In 2007, however, Michi Nakata and co-workers found the nematic and columnar liquid crystal phases in short complementary B-form DNA oligomers with 6-20 base pairs in length. They put the sDNA solutions in gaps of thickness t between glass plates (4 micron < t < 8 micron) and use the depolarized transmission light microscopy to get the optical texture (figure 10).

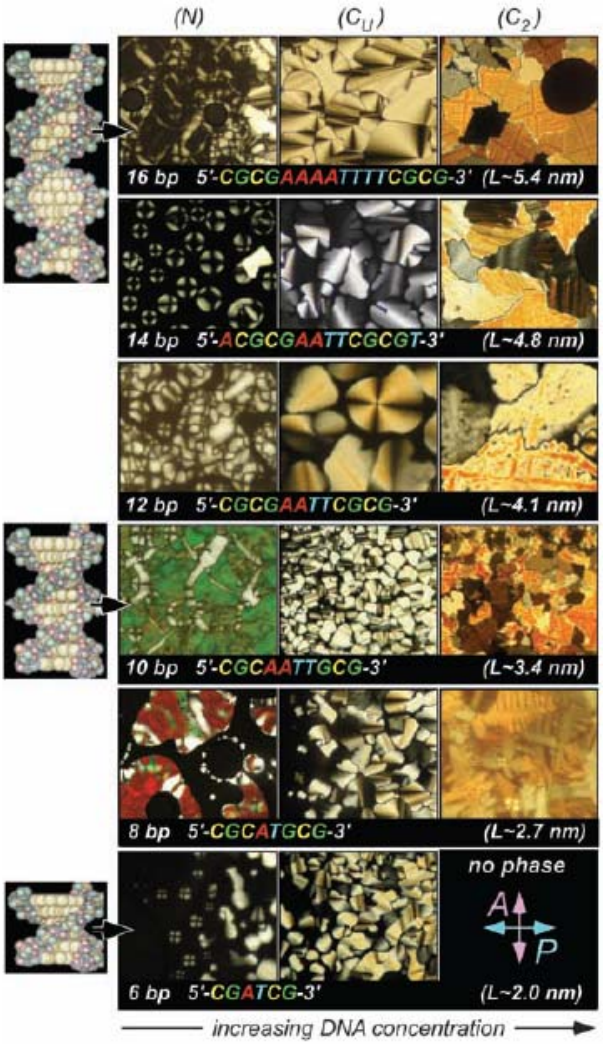


Figure 10. Optical textures of LC phases of a series of solutions of sDNA with different length. The solutions are in the cell that the thickness of the gap is between 4 micron and 8 micron. The black regions are the isotropic phase. The sequence of the phase transition is chiral nematic phase (N*), columnar phase (Cu) and higher-ordered columnar (C2).

Figure 11 shows the process of short DNA forming LC phases. Short DNAs form the cylinders by end-to-end adhesion stacking, then into LC phases.

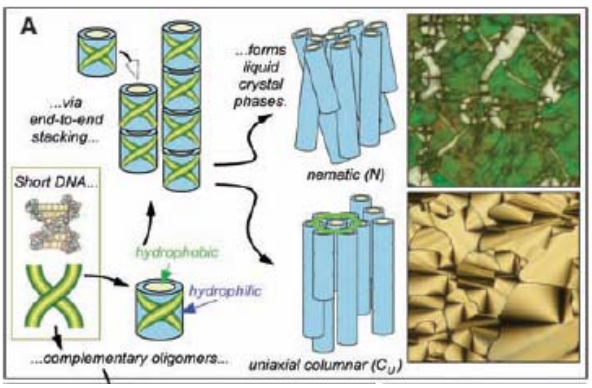


Figure 11. Nano-length B-DNA forms LC phases by end-to-to adhesion and

stacking into units. The chiral nematic phase is formed at lower concentration and the Cu phase at higher concentration.

3 Conclusion

We briefly introduce the structure of DNA, and then present the various liquid crystal phases of DNA of different length ranging from several base pairs to mega-base pairs in detail. We find that the liquid crystalline phases of DNA depend on the concentration and length of DNA.

References:

[1] http://nobelprize.org/educational/medicine/dna_double_helix/readmore.html.

[2] http://en.wikipedia.org/wiki/DNA.

[3] http://www.accessexcellence.org/RC/VL/GG/index.php.

[4] http://www.blc.arizona.edu/molecular_graphics/dna_structure/dna_tutorial.html#Purine.

[5] Maurice Kleman, Oleg D. Lavrentovich. Soft Matter Physics: An Introduction. Springer 1st Ed., 2002.

[6] Alejandro D. Rey. Liquid crystal models of biological materials and processes. Soft matter, 6, 3402-3429 (2010).

[7] Francoise Livolant, Amelie Leforestier. Condensed phases of DNA: structures and phases transitions. Prog. Polym. Sci., 21, 1115-1164 (1996).

[8] Michi Nakata, et al. End-to-End stacking and liquid crystal condensation of 6-20-base pair DNA duplexes. Science, 38, 1276-1279 (2007).

Appendix:

Table 1. Liquid crystalline organization with different types of DNA or polynucleotides in concentrated solutions or in the presence of PEG. [7]

Origin	Length	Ionic environment		Liquid crystalline organization (LC)		References
Linear DNA						
Fowl erythrocyte	unknown	Na^+ up to 1 M		LC, hexagonal packing		Luzzati and Nicolaieff (1959, 1963)[7,8]
Unknown	unknown	Na^+ 0.1 M		cholesteric	$P = 2\ \mu m$	Robinson (1961, 1966)[5,6]
Unknown	~ 200 bp (sonicated)	not precised (moderate salt)	+ PEG	cholesteric	$P = 2.4\ \mu m$	Lerman (1974)[10]
Calf thymus	without sonication	Na^+ 0.1 M		LC (no phase determination)		Iizuka (1977)[85]
Calf thymus	146 bp, 234 bp, 437 bp	Na^+ 0.1 M		LC (no phase determination)		Rill *et al.* (1983)[86]
Calf thymus	150–8500 (sonicated)	K^+ 0.4 M		cholesteric	$P = 2.4\ \mu m$	Livolant (1984)[38]
Calf thymus	150–8500 (sonicated)	K^+ 0.4 M		cholesteric spherulites		Bouligand and Livolant (1984)[37]
Salmon sperm	300–1100 bp (sonicated)	Na^+ + Mg^{++} various	+ PEG	LC (no phase determination)	$Na^+ / Mg^{2+} > 4$ ..... Ψ^- CD $Na^+ / Mg^{2+} < 4$ ..... Ψ^+ CD	Skuridin *et al.* (1985)[55]
Unknown	100 ± 50 bp (sonicated)	Na^+ 0.6 M		cholesteric	$P = 2.5\ \mu m$ $P = 2.7\ \mu m$	Brandes and Kearns (1986)[87]
Calf thymus	146 bp	Na^+ 0.09 M		cholesteric columnar hexagonal (called smectic)		Rill (1986)[88]
Calf thymus	150–8500 bp (sonicated)	K^+ 0.2 M ↗ K^+ 1 M	+ PEG	cholesteric	$P \sim 2\ \mu m$ and $P = 0.2$–$0.4\ \mu m$	Livolant (1986)[35]
Calf thymus	150–8500 bp (sonicated)	K^+ 0.01–0.2 M ↗		columnar hexagonal		Livolant and Bouligand (1986)[64]
Calf thymus	150–4700 bp (sonicated)	K^+ 0.01– 0.2 M ↗		double twist precholesteric stages		Livolant (1987)[63]
Calf thymus	146 bp	Na^+ 0.3 M		cholesteric columnar hexagonal (called higher order)		Strzelecka and Rill (1987)[25]
Calf thymus	150–8500 bp (sonicated)	TE 10 mM K^+ 1 M	+ PEG	cholesteric	Ψ^- CD	Livolant and Maestre (1988)[53]
Various	< 1500 bp	Na^+ 0.3–1 M	+ PEG	cholesteric, nematic	$2\ \mu m <$ $P < 4\ \mu m$	Yevdokimov *et al.* (1988)[21]
Calf thymus	146 bp	Na^+ 0.3 M NH_4^+ 0.25 M		cholesteric (called ''precholesteric'') cholesteric columnar hexagonal (called smectic)		Strzelecka *et al.* (1988)[27]
Calf thymus	146 bp	NH_4^+ 0.25 M		cholesteric	$P \sim 2\ \mu m$	Rill *et al.* (1989)[89]
Calf thymus	500–4500 bp (sonicated)	K^+ 0.2 – 0.4 M K^+ 2 M	+ PEG	cholesteric		Livolant (1989)[46]
Calf thymus	146 bp	Na^+ 0.1 M ↗ NH_4^+ 0.25 M ↗ TE ↗		columnar hexagonal		Livolant *et al.* (1989)[65]
Calf thymus	146 bp	Na^+ 0.01 M		''viscous isotropic'' cholesteric (called ''precholesteric'')		Strzelecka and Rill (1990)[30]
Calf thymus	146 bp	Na^+ 0.01–1 M NH_4^+ 0.3 M (defined and ↗)		cholesteric (called ''precholesteric'') cholesteric columnar hexagonal	$P = 2.1 \pm 0.2\ \mu m$	Van Winkle *et al.* (1990)[28]
Calf thymus	146 bp	Na^+ 0.25 M ↗ NH_4^+ 0.25 M ↗		columnar hexagonal		Livolant (1991a)[66]
pPS-neo plasmid	~ 1200 bp (partially digested by nuclease)	Na^+ 0.3 M + Ca^{++} 0.10 M	+ PEG	cholesteric	$P = 2.3$ mm	Salyanov *et al.* (1991)[90]
Calf thymus	146 bp 150–4700 (sonicated)	Na^+, K^+, NH_4^+ various		double twist precholesteric stages cholesteric columnar hexagonal		Livolant (1991b)[84]
Calf thymus	146 bp	Na^+ 0.01–1 M		''viscous isotropic'' cholesteric (called ''precholesteric'') cholesteric columnar hexagonal (called higher order)		Rill *et al.* (1991)[29]
Calf thymus	146 bp	NH_4^+ 0.25 M Na^+ 0.1 M TE 10 mM		cholesteric	$P = 2.54 \pm 0.9\ \mu m$	Leforestier and Livolant (1993)[36]

Origin	Length	Ionic environment	Liquid crystalline organization (LC)		References
Calf thymus	146 bp 500–5000 (sonicated)	Na^+ 0.25 M and NH_4^+ 0.25 M 0.25 M NH_4^+ ↗	double twist blue phases	"P" = 0.8 μm	Leforestier and Livolant (1994)[59]
Calf thymus	1000 – 13000 bp (sonicated)	Na^+ 0.1 M	LC (no phase determination)		Merchant and Rill (1994)[91]
Unknown	low MW, not precised	Na^+/ Ca^{2+} various + PEG	LC (no phase determination)	Na^+/ Ca^{2+} > 7.5 Ψ^- CD Na^+/ Ca^{2+} < 7.5 Ψ^+ CD	Salyanov *et al.* (1995)[56]
Supercooled pBR 322 linearized by nuclease	< 4363 bp, not precised	Na^+/ Ca^{2+} various + PEG	LC (no phase determination)	Na^+/ Ca^{2+} > 1 Ψ^- CD Na^+/ Ca^{2+} < 1 Ψ^+ CD	Salyanov *et al.* (1995)[56]
Relaxed circular pBR322 linearized by nuclease	< 4363 bp, not precised	Na^+/ Ca^{2+} various + PEG	LC (no phase determination)	Na^+/ Ca^{2+} > 10 Ψ^- CD Na^+/ Ca^{2+} < 10 Ψ^+ CD	Salyanov *et al.* (1995)[56]
Double stranded RNA					
Replicative form of phage f2	~ 1090 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^+ CD	Pyatigorskaya *et al.* (1978)[57]
rRNA	not precised	not precised	cholesteric	P = 3.4 μm	Spencer *et al.* (1962)[92] Spencer and Poole (1965)[93]
Synthetic polynucleotides					
ss poly(A)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	no LC		Iizuka and Yang (1978)[42]
ss poly(U)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	no LC		Iizuka and Yang (1978)[42]
ss poly(C)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	no LC		Iizuka and Yang (1978)[42]
ss poly(G)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	LC (no phase determination)		Iizuka and Yang (1978)[42]
ss poly(I)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	LC (no phase determination)		Iizuka and Yang (1978)[42]
ds poly(A). poly(U)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	cholesteric, nematic	P = 0.8–5 μm*	Iizuka and Yang (1978)[42]
ds poly(A). poly(U)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	cholesteric	P = 0.5 μm Ψ^+ CD	Iizuka (1978)[41]
ds poly(G). poly(C)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	cholesteric, nematic		Iizuka and Yang (1978)[42]
ds poly(C). poly(I)	unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7	cholesteric, nematic		Iizuka and Yang (1978)[42]
ds poly(I). poly(C)	~ 1200 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^+ CD	Pyatigorskaya *et al.* (1978)[57]
ds poly(A). poly(U)	~ 420 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^+ CD	Pyatigorskaya *et al.* (1978)[57]
ds poly(A). poly(dT)	~ 1030 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^+ CD	Pyatigorskaya *et al.* (1978)[57]
ds poly[d(A-T)]. poly[d(A-T)]	~ 272 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^- CD	Pyatigorskaya *et al.* (1978)[57]
ds poly(dG). poly(dC)	~ 788 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^- CD	Pyatigorskaya *et al.* (1978)[57]
ds poly(dA). poly(dT)	~ 636 bp	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^- CD	Pyatigorskaya *et al.* (1978)[57]
ds poly(A). poly(U)	~ 170 bp	not precised	double twist cholesteric		Senechal *et al.* (1980)[94]
ds poly(I). poly(C)	450–2000 bp	Na^+ 0.3–1.3 M + PEG	LC (probably nematic/ cholesteric)	$\theta \leq 68°C$.....Ψ^- CD $\theta \geq 73°C$.....Ψ^+ CD	Skuridin *et al.* (1988)[58]
ts poly(A). 2poly(U)	not precise	Na^+ 0.3 M + PEG	LC (no phase determination)	Ψ^+ CD	Pyatigorskaya *et al.* (1978)[57]

Origin		Length	Ionic environment		Liquid crystalline organization (LC)		References
ts poly(A). 2poly(U)		unknown	Na^+ 0.08 M + 0.2 M Na citrate, pH 7		cholesteric, nematic	P = 2–10 μm*	Iizuka and Yang (1978)[42]
Oligonucleotides $[d(CGCGAAT^*T^*CGCG)]_2$		Na^+ not precised	LC (no phase determination)				Alam and Drobny (1990)[95]
Guanosine derivatives d(pG)		—	$NH4^+$		cholesteric and hexagonal	left-handed cholesteric	Spada *et al.* (1988)[96]
d(GpG)		—				left-handed cholesteric	Mariani *et al.* (1989)[97]
d(GpGpG)		—				right-handed cholesteric	Bonazzi *et al.* (1991a)[98]
d(GpGpGpG)		—				right-handed cholesteric	Bonazzi *et al.* (1991b)[99]
d(GpGpGpGpG)		—				right-handed cholesteric	
d(GpGpGpGpGpG)		—				right-handed cholesteric	
Circular supercoiled DNA							
Monomers	PUC8	2717 pb	Na^+ 0.1 M		LC (maybe hexagonal)		Torbet and DiCapua (1989)[80]
	PKS 414	2671 pb					
Dimers	PUC8	5434 pb					
	PKS 414	5342 pb					
pPS-neo		unknown	Na^+ 0.3 M + Mg^{++}	+ PEG	LC (no phase determination)		Salyanov *et al.* (1991)[90]
pBR322		4363 bp	0.01 M				
pGC 20		2704 bp					
Bluescript		2960 bp	0.8 M NaCl 0.01 M MgCl2		LC (no phase determination)		Reich *et al.* (1994)[79]

*P varies with the DNA concentration.