Magnetic-field alignment of cholesteric liquid-crystalline DNA

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In moderately high fields (3.5 and 6.4 T) the cholesteric phase of liquid-crystalline DNA aligns with its twist axis parallel to the applied field. Thus when the field is applied in the plane of a thin sample, the well-known ''fingerprint'' texture is observed with striations of width ~1.1 μm orienting perpendicular to the field. In a 9.4 T field, the cholesteric aligns, still with the twist axis on average parallel to the field, but locally distorted into longer wavelength stripes. The orientation of these larger stripes is parallel to the magnetic field, thus perpendicular to the fingerprint striations. The field stabilizes the stripes, and upon removal from the magnet, the stripes anneal away. The time scale for relaxation of the stripes to an aligned fingerprint texture is several days. Defect sites present in the field-stabilized stripe structure persist and become quite mobile (in the presence of a concentration gradient) after the stripes have annealed away. [S1063-651X(97)05904-7]

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INTRODUCTION

The morphologies of liquid-crystalline samples as seen in a polarizing microscope are revealing of the underlying intermolecular order. Polymer liquid crystals exhibit some different morphologies compared to small molecule liquid crystals. These differences are usually seen when the sample is aligned in a field and are due to the larger axial ratios, leading to very anisotropic orientational elasticities, found in the long polymer liquid crystals [1]. Most liquid crystals exhibit a positive diamagnetic anisotropy, and thus align with the long molecular axis (on average) parallel to an applied magnetic field. We have investigated the morphologies that are observed in samples of 50-nm DNA fragments in aqueous solution. DNA has a negative diamagnetic anisotropy, and thus behaves differently from other polymer and small molecule liquid crystals when aligned by magnetic fields. Many of the morphologies observed in the liquid-crystalline DNA (lc DNA) samples are easy to interpret when compared with those observed in other liquid crystals. Some of the simpler to interpret morphologies were reported previously in a study of the dependence of helical pitch on DNA concentration [2]. Samples tending to align with the twist axis perpendicular to the plates form terraces analogous to ''Grandjean planes'' [3]. Details of the unwinding of the cholesteric twist as the hexagonal columnar phase was approached were discussed in a simulation of that unwinding [4]. Reported here are observations of morphologies that appear in the cholesteric phase of lc DNA when it is subjected to moderately high magnetic fields. A region of field-stabilized birefringent stripes was observed [5] that then annealed into a fingerprint texture. These stripes are very similar to chevrons observed by Bouligand in methoxy-benzylidene-butyl-aniline (MBBA) doped with chiral enantiomorphs to produce twisted nematics [6]. As the columnar phase approached, mobile defects were observed that apparently unwind the cholesteric twist. The growth of the columnar phase is quite anisotropic relative to the orientation of the molecules.

DNA fragments in aqueous ionic solution form a variety of lyotropic liquid-crystalline phases [7]. Cholesteric phases were first observed in DNA liquid-crystalline systems by Robinson in the early 1960s [8]. Maret and co-workers [9] and Iizuka and co-workers [10] studied cholesteric liquid crystals formed by polydisperse polyribonucleic acid strands. Livolant and co-workers [11,12] reported that DNA, and two other lyotropic polymer solutions, poly-benzyl-L-glutamate and xanthan, form hexagonal liquid-crystalline phases at high density. Rill and co-workers developed the techniques for obtaining concentrated solutions of 50 nm by 2.5-nm DNA fragments with small polydispersity observed via NMR [13] and depolarized optical microscopy [7]. A twisted nematic with very long pitch of the twist (called *precholesteric*) exists for $C_D<220$ mg/ml. A cholesteric phase with pitch 2.2 μm nucleates at $C_D\approx160$ mg/ml. The cholesteric unwinds in the range 330–400 mg/ml prior to formation of a hexagonal columnar phase at higher C_D [2]. Podgornik and co-workers have recently studied the phase behavior of concentrating DNA fragments under osmotic stress to elucidate better the thermodynamics of the phases and the transition [14].

MAGNETIC PROPERTIES

Liquid crystals are composed of anisotropic constituents and are characterized by anisotropic material parameters. DNA has an anisotropy in diamagnetic susceptibility, $\chi_a=\chi_\parallel-\chi_\perp<0$. If the DNA fragments are modeled as rods, the magnetic field exerts a torque tending to align the rods perpendicular to the field [9,15–17]. The sign of the anisotropy in χ is a consequence of the orientation of the polycyclic aromatic ring structure of the bases, which lie perpendicular to the DNA helix axis. In general, a liquid-crystal phase will align if the free energy cost to reorient the liquid crystal is less than the energy reduction for alignment in the magnetic field $F_{\rm mag}=-(\frac{1}{2})\chi_a(\mathbf{n}\cdot\mathbf{H})^2$, where $\mathbf{H}$ is the magnetic field and $\mathbf{n}$ is the symmetry axis for molecular orientation (i.e., the nematic director).

Under certain conditions, nematics with positive diamagnetic anisotropy undergo reorientation at a critical magnetic field [18]

$$H_c = \frac{\pi}{d}\sqrt{K_i/\chi_a},$$

where K_i is the elastic constant for splay, twist, or bend depending upon which elasticity is distorted. This simple expression is only applicable when there is strong anchoring of the liquid-crystal orientation at the boundaries of a rectangular prism, the initial director orientation is uniformly parallel or perpendicular to the boundaries, and the orientational elastic constants are not too anisotropic. Away from the boundaries, the director rotates uniformly from the surface orientation to parallel (or antiparallel) to the field direction. If, in neighboring regions, domains nucleate that have rotated with opposite senses (one to become parallel, the other antiparallel), then a line defect is formed at the boundary between the two regions. Upon removal from the magnetic field, this high-energy defect may move, increasing the volume occupied by one sense of rotation at the expense of the other.

In the case of DNA fragments in aqueous solution, all the molecules can orient with their long axes perpendicular to an applied field (thus minimizing the free energy) by rotating the cholesteric twist axis parallel to the field. Again there are two possible senses of rotation to align the preferred axis (in this case twist rather than director), but a line defect is not required to bound neighboring regions of opposite sense. Thus one might expect to see uniform fingerprint textures for all aligning fields. This is observed for the lower field values in DNA [2,5] and has recently been observed in suspensions of cellulose crystallites [19]. In order to understand the birefringent stripes observed in high fields the Euler-Lagrange equations derived from the Frank free energy were analyzed [20].

EXPERIMENT

DNA with a most probable length of approximately 50.0 nm was isolated from calf thymus chromatin and purified as previously described [13]. The DNA was concentrated by dialysis first against buffer at reduced pressure and then by equilibration with dry Sephadex beads as described in Ref. [2]. The resulting solutions have supporting electrolytes (primarily NaCl) in the concentration range (0.01–1.0) M. Routinely, a 10 μl lc DNA sample was placed between microscope coverslip and slide. The edges of the coverslip were sealed to the slide with a solubilized poly-methylmethacrylate (PMMA) resin. Some samples were made with a 2–3-mm gap in the PMMA, thus allowing evaporation of water from the DNA solution. These have been called ''controlled drying'' samples. Samples were examined under a polarized light microscope prior to any alignment studies. Magnetic-field alignment studies were done in four fixed fields formed by a 1.1 T electromagnet, and 150 MHz (3.5 T), 270 MHz (6.4 T), and 400 MHz (9.4 T) NMR magnets. No reorientation of any sample was observed in the electromagnet. The cholesteric phase reoriented in all fields of $\geqslant$3.5 T. All samples were oriented with the long axis of the slide parallel to the field to fit in the bore of the NMR magnets.

Polarized light microscopy is useful for studying DNA liquid crystals since the samples exhibit uniaxial birefringence. This is an observable manifestation of the long-range order in liquid crystals and the excess polarizability parallel to the DNA rods. Samples were examined utilizing a Nikon Optiphot-pol microscope illuminating a film camera or an Olympus polarizing microscope, illuminating a color video camera. Images were recorded on a Panasonic video disk recorder that was controlled by a Silicon Graphics IRIS workstation. Gross sample morphology was recorded at low magnification using 4× and 10× objectives. For some observations higher magnification was available using a 40× objective. In some cases a 530 nm first-order retardation plate was used to determine the sign of the birefringence relative to the extinction direction.

RESULTS

We report here a series of observations of optical morphologies spontaneously formed in lc DNA samples, primarily in the cholesteric phase. Figure 1 shows photomicrographs from three separate samples, each of which show cholesteric phase in contact with the higher-density hexagonal columnar phase. These samples were prepared by ''controlled drying,'' hence there is a decreasing concentration gradient perpendicular to the boundary of the high-density phase. They should therefore be considered as samples very far from equilibrium. Figure 1(a) shows a series of fields of distinct intensities from top down, which are all cholesteric phases of increasing C_D. The columnar phase appears as the mottled region at the bottom of the picture. In this case, the DNA rods are aligned parallel to the glass boundaries in the cholesteric phase, and the twist axis is perpendicular to the photograph plane. The total twist decreases with increasing C_D from top to bottom, in quantized steps of π radians at the boundaries between fields of distinct intensities. This sample was not aligned in a magnetic field. Monte Carlo simulation has been used to understand this terraced morphology in detail [4]. The growth of the columnar phase, as seen at the boundary, is reminiscent of dendritic crystalline growth. Figure 1(b) shows a sample that was placed in a 6.4 T magnetic field with the dominant direction of growth of the new phase (and therefore ∇C_D) almost parallel to the field direction. The fingerprint texture is seen clearly as striations in the upper part of the photograph. This is observed when the twist axis is in the sample plane and the molecular orientation rotates in (birefringent) and out (not birefringent) of the plane of the sample. Remnant cholesteric striations can be seen between the dendritic fingers of columnar phase. The columnar phase grows more rapidly parallel to the twist axis rather than perpendicular to it. This is consistent with unwinding of twist to allow the DNA molecules to lie parallel to their neighbors as the columnar phase grows. The preferred growth direction for the columnar phase is therefore to form new columns laterally, instead of elongating columns.

Figure 2 shows two samples in which the columnar phase grew out of the cholesteric by nucleation at multiple sites. The samples were each completely sealed with PMMA and therefore dried very slowly. Eventually enough water evaporated through the PMMA to bring the entire sample (in quasiequilibrium) to the nucleation point for the columnar phase. The sample shown in Fig. 2(a) was prepared in the absence of a magnetic field and shows the morphology observed for

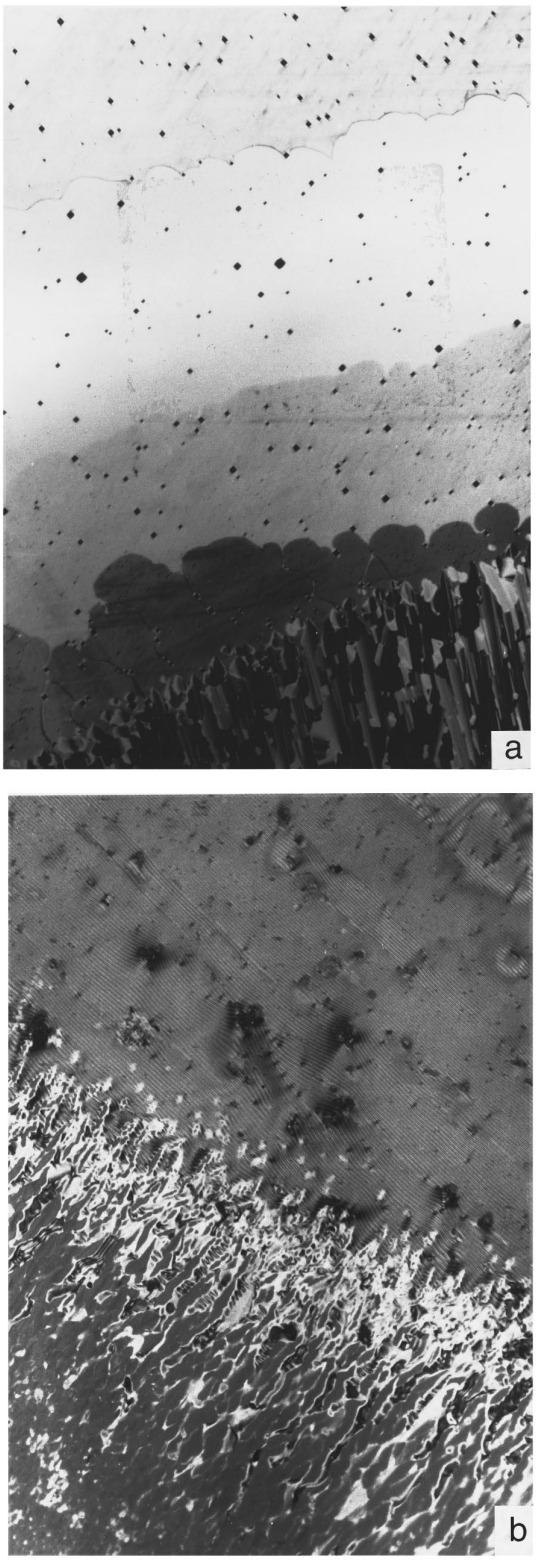

FIG. 1. (a) Two phases of liquid-crystalline DNA, the cholesteric and the columnar, are shown in the presence of a concentration gradient. The columnar is the mottled region at the bottom of the picture. The different intensity regions are all cholesteric phases of different twist (therefore different birefringence) between the slides. (b) The same two phases are seen, cholesteric and columnar, but now the cholesteric twist axis has been reoriented through a Freedericksz transition to be perpendicular to the average orientation of the striations in the upper part of the picture.

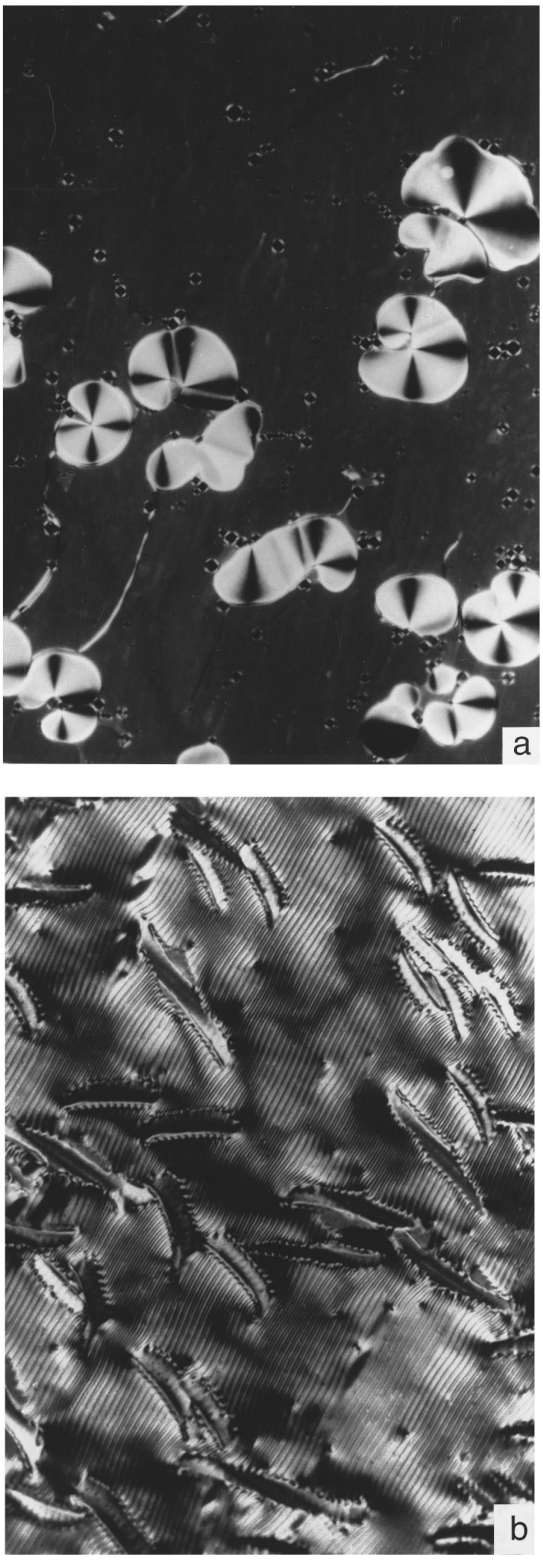

FIG. 2. (a) Two liquid-crystalline phases of DNA are shown in a sample of uniform concentration. The dark background is the cholesteric phase with twist axis perpendicular to the photograph. The brighter regions with extinction brushes are the columnar phase, growing slowly as an almost circular domain. (b) The same two phases at a uniform concentration, but aligned in a magnetic field of 6.4 T. The anisotropic growth of the narrow nonstriated columnar phase is seen clearly in contact with the cholesteric fingerprint texture.

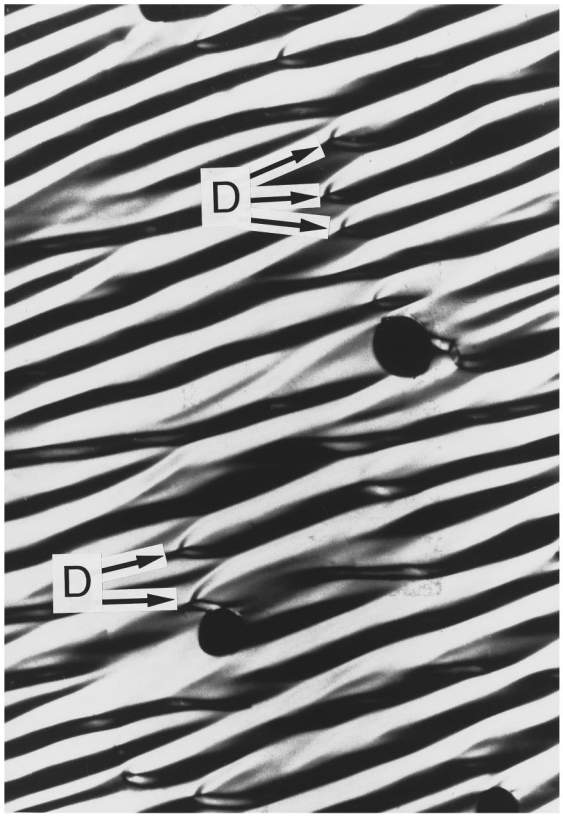

FIG. 3. A cholesteric lc DNA sample aligned in a 9.4-T magnetic field directed parallel to the long axis of the birefringent stripes. The characteristic width of these stripes ranges from 5 to 8 μm. The fingerprint texture is not resolved in this image, but does appear at higher magnification as lines perpendicular to the axis of these stripes.

planar alignment of the DNA rods relative to the glass bounding surfaces. The dark background actually appears blue and is the cholesteric phase. The total molecular twist within this dark region is approximately constant as is the thickness; thus the linearly polarized light that entered the sample from below emerges elliptically polarized and passes the crossed analyzer. This dark cholesteric is equivalent to the region closest to the columnar phase shown in Fig. 1(a). The brighter regions with dark brushes are regions of columnar phase. The DNA rods condense into the columnar phase tangential to the curvature of the growing columnar domain. Figure 2(b) shows the growth of the high-density phase in an aligning magnetic field. The magnetic-field direction is perpendicular to the striations. The columnar phase are those elongated, narrow regions that have no striations. The growth is similar to that seen in the dendritic growth of Fig. 1(b).

Figure 3 is a photomicrograph of cholesteric DNA at lower C_D than the samples discussed above, well below the high-density transition. A 9.4-T magnetic field applied parallel to the average orientation of the birefringent stripes resulted in this morphology. The average width of stripes ranges from 5 to 8 μm. At higher magnification, it was possible to see the ''fingerprint'' structure with its 2.2-μm pitch within and perpendicular to particular stripes. Thus the sample remains locally cholesteric, but exhibits a longer

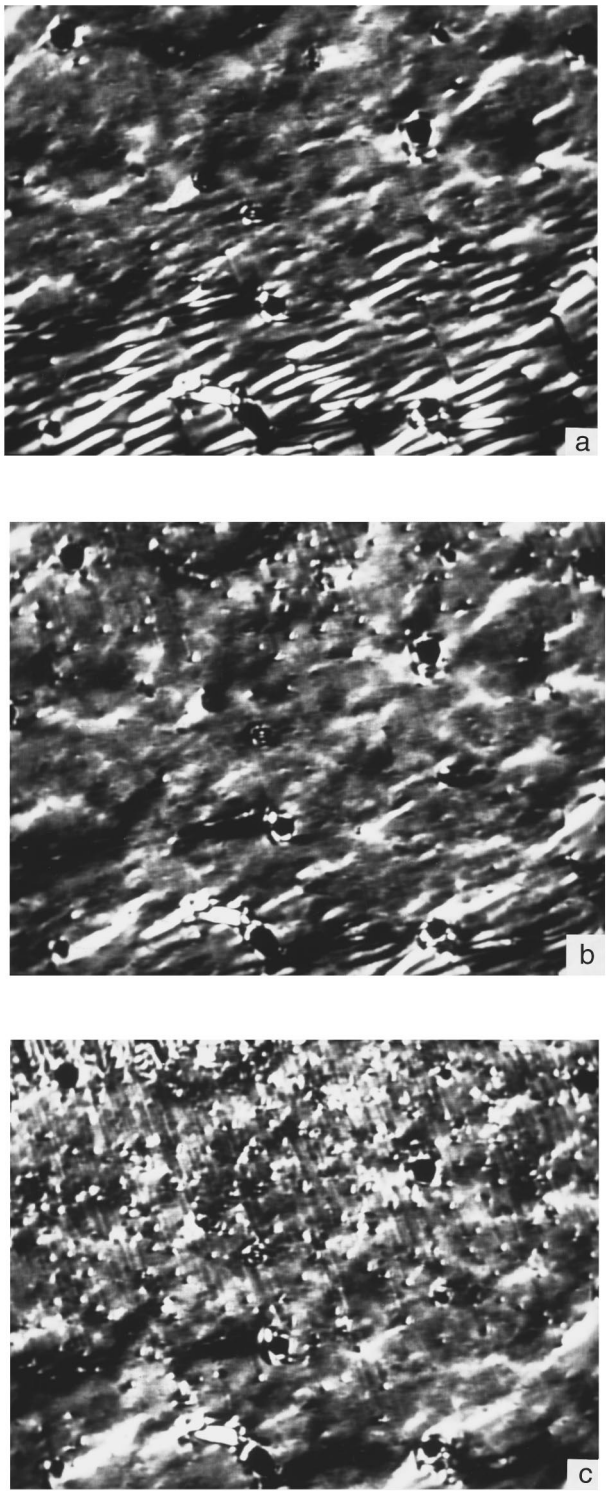

FIG. 4. A sequence of three images separated in time by 1000 min is shown. These were taken from a video display record of the controlled drying of a cholesteric lc DNA sample that had been aligned in the 9.4-T field. (a) The birefringent stripes can be seen at the bottom of the image. The field direction was parallel to the average orientation of those stripes. (b) A few remnant birefringent stripes are still seen at the bottom of this image, along with several defect points near the top of the image. The defects have moved, leaving linear trails that follow fingerprint striations. (c) There are many more defects in this image and near the top the columnar phase is just appearing.

wavelength periodicity perpendicular to the direction of the magnetic field. This stripe structure was never observed in samples aligned in lower-field magnets. This sample was further investigated with a 530-nm first-order retardation plate in the optical path. It showed that the stripes consist of two regions (yellow and blue) of different sign of birefringence, while, for example, the isotropic bubbles are red (no birefringence). Bouligand interpreted what appears to be the same morphology. That phenomenological description suggests the cholesteric twist axis became tilted out of the plane of the slide and splayed on either side of the centerlines that include the defect sites [2]. By numerically solving the Euler-Lagrange equations resulting from minimization of the free energy [20], it has been shown that the stripe morphology arises from an oscillatory distortion of the orientation field. The simulation indicates that the birefringent stripes are stabilized at high enough magnetic field when fluctuations away from the minimum energy twisted state are present. Realizing that the director twist axis is, on average, parallel to the stripe axes, the distortions in director field perpendicular to the stripe axes are a combination of splay and bend, being completely splay where the director is on average perpendicular to the plane of the photograph, and completely bend where the director is parallel to the plane of the photograph. The amplitude of this field-induced director distortion decays to zero in short distances in low magnetic fields. In higher fields the amplitude is large and is stabilized by damping due to nearest-neighbor restoring forces. The periodicity of the distortion decreases with increasing field, becoming comparable with the sample thickness at magnetic fields of $\sim$10 T.

Upon removal from the magnetic field, the long-wavelength striped structure anneals away leaving the ''fingerprint'' texture. The time scale for this ranges from 10 h to several days. Even after the stripes anneal away, the points where stripes of opposite sign of birefringence met (labeled with *D*'s in Fig. 3) are discernable in the sample. These sites seem to scatter light strongly and persist for long times. As the columnar front continues to advance, these sites become mobile. Figure 4 shows a sequence of images of the same area on a microscope slide, which is evolving in time as water evaporates. The time between images is 1000 min. Figure 4(a) shows primarily uniform cholesteric, with an area of birefringent stripes at the bottom and a few mobile defects as bright spots near the top. Figure 4(b) shows more defects and that the region of stripes has annealed into a uniform fingerprint texture. The fingerprint is oriented with the twist axis parallel to the average long axis of the birefringent stripes. Figure 4(c) shows many more defect sites, and at the top a highly birefringent area, that is the columnar phase. The direction of **H** was perpendicular to the gradient in concentration, thus the cholesteric ''fingerprint'' striations are parallel to ∇C_D. Most of the defect sites move away from the advancing columnar front while a few move towards the front. Those that move towards the front all are born from a single stationary site that splits in two. One of the pair moves along the ''fingerprint'' striations away from the front, while the other moves towards the front. The velocities of the mobile sites are similar to but larger than the velocity of the advancing front, as large as twice the speed of the front (which is determined by the evaporation rate and is about 10^{-6} m/min).

We believe the mobile sites are defects in the cholesteric twist periodicity, which occur as the twist is forced to unwind to allow the formation of the columnar phase. This type of defect was analyzed in detail in the planar geometry [not magnetically aligned, Fig. 1(a)] [4]. In that case, defect lines advanced at the same speed as the columnar phase boundary, and there never appeared defect lines that moved towards the columnar boundary. These defect lines ran across the entire width of the sample, bounding regions of different total twist. In the case illustrated in Fig. 4, the defects are confined to a volume comparable to the thickness of the sample cubed, representing the removal or addition of an integral number of one-half twists.

We have reported a variety of optical morphologies observed in the cholesteric and columnar phases of DNA liquid crystals aligned in moderately high magnetic fields. DNA has a negative diamagnetic anisotropy and, to our knowledge, very few materials with such an anisotropy have been studied [19]. The cholesteric fingerprint texture has been observed previously. The observation of birefringent stripes [Figs. 3 and 4(a)] imposed by the 9.4-T field is unique [5]. The observations that the birefringent stripes anneal into a relatively uniform fingerprint texture and of mobile defects parallel to the fingerprint striations have, to our knowledge, not been previously reported. The birefringent stripes are interpreted as a magnetic-field-induced distortion calculated separately [20]. The mobile defects are interpreted as defects in twist periodicity seen as the twist unwinds with increasing concentration of DNA prior to the formation of the columnar phase. This observation complements the observation for planar geometry reported elsewhere [4].

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[1] F. Lonberg and R. B. Meyer, Phys. Rev. Lett. **55**, 718 (1985).
[2] D. H. Van Winkle, M. W. Davidson, W. X. Chen, and R. L. Rill, Macromolecules **23**, 4140 (1990).
[3] D. H. Van Winkle, M. W. Davidson, and R. L. Rill, J. Chem. Phys. **97**, 5641 (1992).
[4] A. Chatterjee and D. H. Van Winkle, Phys. Rev. E **49**, 1450 (1994).
[5] D. H. Van Winkle, M. W. Davidson, and R. L. Rill, in *Physical Phenomena at High Magnetic Fields-Proceedings*, edited by E. Manousakis, P. Schlottmann, P. Kumar, K. S. Bedell, and F. M. Mueller (Addison-Wesley, Redwood City, CA, 1992), p. 469.
[6] Yves Bouligand, J. Micros. **17**, 145 (1973).
[7] T. E. Strzelecka, M. W. Davidson, and R. L. Rill, Nature **331**, 457 (1988).
[8] C. Robinson, Tetrahedron **13**, 219 (1961).

[9] G. Maret, M. Schickfus, A. Mayer, and K. Dransfeld, Phys. Rev. Lett. **35**, 397 (1975); G. Maret and K. Dransfeld, Physica **86-88B**, 1077 (1977); E. Senechal, G. Maret, and K. Dransfeld, Int. J. Biol. Macromol. **2**, 256 (1980).

[10] E. Iizuka, Polym. J. **9**, 173 (1977); E. Iizuka and Y. Kondo, Mol. Cryst. Liq. Cryst. **51**, 285 (1979); E. Iizuka, Polym. J. **10**, 237 (1978); **10**, 293 (1978); J. Appl. Polym. Sci. **41**, 131 (1985).

[11] F. Livolant and Y. Bouligand, J. Phys. (Paris) **47**, 1813 (1986).

[12] A. M. Livolant, F. Levelut, J. Doucet, and J. P. Benoit, Nature **20**, 724 (1989).

[13] T. E. Strzelecka and R. L. Rill, J. Am. Chem. Soc. **109**, 4513 (1987).

[14] See, for example R. Podgornik, H. H. Strey, D. C. Rau, and V. A. Parsegian, Biophys. Chem. **57**, 111 (1995), and references therein.

[15] R. L. Rill, F. Livolant, H. C. Aldrich, and M. W. Davidson, Chromosoma **98**, 280 (1989).

[16] E. Senechal, G. Maret, and K. Dransfeld, Int. J. Biol. Macromol. **2**, 256 (1980).

[17] R. Brandes and D. R. Kearns, Biochemistry **25**, 5890 (1986).

[18] R. B. Meyer, Appl. Phys. Lett. **12**, 281 (1968), and, in the form used here, P. G. DeGennes, *The Physics of Liquid Crystals* (Oxford University Press, Clarendon, 1979), p. 90.

[19] J.-F. Revol, L. Godbout, X.-M. Dong, D. G. Gray, H. Chanzy, and G. Maret, Liq. Cryst. **16**, 127 (1994).

[20] A. Chatterjee, and D. H. Van Winkle, following paper, Phys. Rev. E **55**, 4360 (1997).