DNA that is dispersed in the liquid crystalline phases of phospholipids is actively transcribed†

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We report that a 4.3 kbp linearised T7 DNA plasmid is actively transcribed when it is dispersed in the hexagonal liquid crystalline phase of dioleoylphosphoethanolamine (DOPE).

Recently we reported that cell nuclei, stripped of their nuclear membranes (so-called naked nuclei), contain substantial amounts of lipids, typically in the range 5–14% of the total cell lipid content.[1] This pool of endonuclear lipids was found by mass spectrometry to have a distinctive composition, which is characterized by a higher incidence of glycerophospholipid species that bear saturated acyl or alkenyl chains than is found in exonuclear or nuclear membrane lipids. Our studies, together with those of others, have shown that cell nuclei possess the capacity to synthesize lipids independently of the biosynthetic pathways that occur outside the nucleus.[1,2] While there is now a compelling body of evidence that suggests links between endonuclear lipid composition and the functional competence of nuclei,[3] the precise role that lipids play in nuclear function remains unclear. We were particularly intrigued by the fact that, from conventional histological studies, the endonuclear lipid pool does not appear to be organized into membranous or vesicular structure and is not associated with invaginations of the nuclear membrane. In view of the typical concentrations of endonuclear lipids that we have observed, together with the limited amount of water that is present in cells, we postulated that endonuclear lipids might form self-organized mesoscale architectures, possibly related to one of the lyotropic liquid crystalline phases that have been widely documented for these types of amphiphiles in the presence of DNA and histones.[4–8] This possibility is supported by occasional reports of endonuclear membranous structures that have morphologies analogous to those of bicontinuous lyotropic cubic phases.[9]

In order to test this hypothesis we used polarized light microscopy to investigate naked nuclei, isolated from an asynchronous culture of H2B-GFP expressing HeLa cells using detergent-mediated lysis as described previously.[1] The occurrence of anisotropic phases, such as liquid crystalline phases, within the nuclei might be expected to result in birefringence, which would be seen as an optical texture under crossed polaroids. Naked nuclei dispersed in excess water showed no evidence of birefringence. However, naked nuclei that had been freeze-dried were characterized by a diffuse birefringence. We found that the naked nuclei retained their integrity throughout the lyophilization process and were observable as distinct 11.9 (±2.2) μm diameter spherical objects, at high magnification. Rehydration of the lyophilized naked nuclei enhanced considerably the birefringent appearance of the preparations and the optical textures were reminiscent of those reported for the liquid crystalline phases formed by phospholipids (Fig. 1). Although the optical texture exhibited by the naked nuclei is too diffuse to allow phase identification, the occurrence of distorted air bubbles and the feathery diffuse texture are remarkably similar to those that are often observed in inverse topology hexagonal phases that are in contact with excess water.

We note that the organization of genomic material into liquid crystalline phases has been described previously. Small angle X-ray diffraction studies of *E. coli* JM109 cells that carry a high copy-number of the non-nucleosomal Blue Script plasmid have shown weak Bragg peaks at 49.1 Å and 51.5 Å from these organisms.[10] The origin of the Bragg peaks has been ascribed to the presence of a columnar lyotropic phase arising from the high volume fraction of the plasmid DNA. The ability of highly condensed DNA to form liquid crystalline phases both *in vitro*[11–13] and *in vivo*, for example in the nuclei of the dinoflagellate *Prorocentrum micans* as well as in mammalian spermatozoids, has also been documented.[14,15]

We postulated that if the cell nucleus has a liquid crystalline mesostructure that is the result of lipid self-assembly, then the normal nuclear functions (and in particular transcription) should be able to occur within the orientationally and spatially ordered environment that is characteristic of lyotropic liquid crystalline phases. To test this hypothesis we conducted a series of experiments using a linearised T7 luciferase plasmid (*lin*T7-Luc, 4331 bp) as a model genome dispersed within the inverse hexagonal phase of DOPE. In these experiments the linearised plasmid was prepared as a solution in nuclease-free water which was used to rehydrate a lyophilized preparation of DOPE in isotonic saline. At 37 °C the inverse hexagonal phase of DOPE has a gel-like consistency. The transcription competence of the H_{II}-DOPE/*lin*T7-Luc mixtures was

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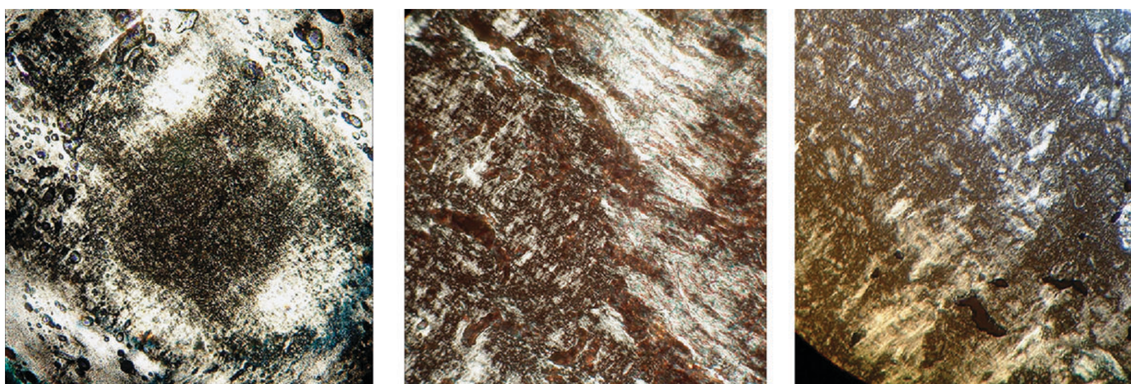

Fig. 1 Optical micrographs of rehydrated naked nuclei from HeLa cells viewed under crossed polaroids, showing birefringent textures at 37 °C. (left) Preparation obtained using Triton X-100, (middle) preparation obtained using β-decylmaltopyranoside, (right) H_{II} phase formed by DOPE.

quantified using the Megascript© Transcription Assay, which contains the appropriate RNA polymerase as well as free bases. The assay mixture was layered onto the H_{II}-DOPE/*lin*T7-Luc mixture and incubated for 2 h. RNA transcript was purified from aliquots of the supernatant using NucAway© spin columns. The RNA content of the eluted solutions was quantified using a NanoDrop Technologies ND-1000 spectrophotometer and visualized using agarose gel electrophoresis.

Our results (Fig. 2) show unambiguously that linearised T7 plasmid contained within the inverse hexagonal lyotropic phase is transcriptionally active. Optical density data indicated an mRNA concentration of 192 ± 54 ng μl^{-1} in the supernatant of the H_{II}-DOPE/*lin*T7-Luc mixture. This compares with 60 (±10) ng μl^{-1} for the negative control sample (isolate from mixtures containing DOPE and no T7 DNA) and 452 (±25) ng μl^{-1} for the positive control (isolates from linearised T7 in the absence of DOPE). The production of mRNA from the H_{II}-DOPE/*lin*T7-Luc mixture was confirmed by using a TnTQuick© coupled transcription/translation system, which showed the presence of luciferase.

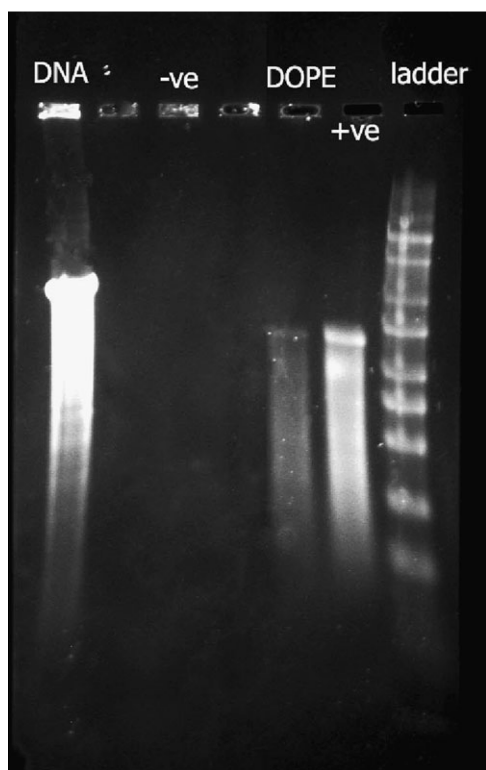

Fig. 2 Agarose electrophoresis gels showing transcription in H_{II}-DOPE/*lin*T7 mixtures with production of RNA that corresponds to the 4000 bp component of the standard RNA ladder. Lane 1: linearised T7 DNA. Lane 3: negative control (DOPE with no DNA). Lane 5: RNA from H_{II}-DOPE/*lin*T7 mixture. Lane 6: positive control, RNA from *lin*T7. Lane 7: RNA size marker.

The observation of transcription from a linearised plasmid confined to the aqueous domains of an inverse hexagonal phase is counter-intuitive because it might have been assumed that the structure of the hexagonal phase would not be able to accommodate the transcription machinery, or that it would present a barrier to the products of transcription diffusing out of the phase. To investigate the possibility that the transcription observed might have been due to DNA that leached out of the H_{II} phase, resulting in transcription occurring in the supernatant phase, we conducted an experiment in which the transcription mixture, including the appropriate RNA polymerase, but lacking bases, was added to the liquid crystalline gel formed by DOPE and incubated for three hours. An aliquot of the supernatant phase was then removed and the free bases were added to this. If DNA had leached from the H_{II} phase, the result would have been transcript RNA. Our experiments showed no sign of transcript RNA (the optical density of supernatant corresponded to a value of 54 ng μl^{-1} RNA, while that of the positive control sample was 472 ng μl^{-1}), confirming that transcription does not occur in the supernatant solution. Further evidence in support of the conclusion that DNA is retained within the liquid crystalline phase comes from experiments using binary mixtures of DOPE and the cationic lipid dioleoyltrimethylammonium propane (DOTAP) that are in an H_{II} phase. In these systems it is expected that the cationic charge within the phase will provide an anchor for the DNA. We observed no significant differences in the rates of transcription between the H_{II}-DOPE/*lin*T7-Luc system and the H_{II}-(DOPE + DOTAP)/*lin*T7-Luc system in which DOTAP is present at 5 mol% (data not shown).

Small angle X-ray diffraction studies of the H_{II}-DOPE/*lin*T7-Luc system show that the lattice parameter of the H_{II}

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phase for the mixture (47.2 Å) is effectively identical to that of the H_{II} phase of pure DOPE made up with saline (47.3 Å). This invariance is not surprising when considering the relative amounts of DNA and lipid. Assuming that the linearised T7 plasmid resides within the aqueous channels of the phase, we calculate that the DNA occupies only 1 in 760 to 1 in 560 of the channels. This calculation is based on an estimate of the T7 length (4331 bp $\times$ 3.4 Å bp^{-1}), the diameter of the double helix ($\sim$20 Å) and the area per head group of DOPE (65 $Å^2$–48 $Å^2$). We also conducted experiments using salmon sperm DNA ($\sim$200 bp long); these show that the lattice parameter for the H_{II} phase of DOPE, in which each channel is, on average, occupied by a DNA molecule, is 47.2 Å. These observations are consistent with the DNA being localized within the aqueous channels of the H_{II} phase, in a manner that is analogous to that described in previous work for mixtures of phosphatidylcholine lipids and calf thymus DNA, which have a repeat distance of 60 Å.[4]

In view of the evidence we obtained for transcription in the H_{II}-DOPE/*lin*T7-Luc system, we carried out an experiment to assess whether the transcription machinery can indeed gain access to the plasmid within the H_{II} phase. A sample of DOPE in its H_{II} phase, but without DNA, was placed in contact with the transcription assay mixture and incubated at 37 °C for 2 h. Aliquots of supernatant were taken and linearised T7 DNA was added; the mixtures were then incubated for a further 2 h. The results show only low levels of transcription in the system that had been incubated with the H_{II} phase (RNA concentration is 67 ng μl^{-1} compared with 464 ng μl^{-1} for the positive control). This suggests that key components of the transcription system are taken up readily into the H_{II} phase when they come in contact with it, thereby reducing their concentration in the supernatant and hence causing the low levels of transcription.

Our discovery that a model DNA 'genome' contained within an inverse topology hexagonal phase is transcriptionally active, together with the observation of birefringence in preparations of naked nuclei, raises the intriguing possibility that endonuclear lipids might form organized mesostructures within the nucleus *in vivo*. How components of the transcription machinery would operate within such a structured environment is yet to be ascertained, as is the role, if any, of the meso-architecture of the phase in controlling transcription. The observation that the nanoarchitecture of complexes of a zwitterionic lipid and DNA changes from lamellar to rectangular columnar over an extended period suggests the possibility that analogous temporal changes in mesostructure might be related to genome function.[8] From the perspective of Synthetic Biology, our discovery opens up new possibilities for the engineering of synthetic nuclei as chemosynthetic modules that can be interfaced with microfluidic devices for the production of proteins or for otherwise encoding chemical information.

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