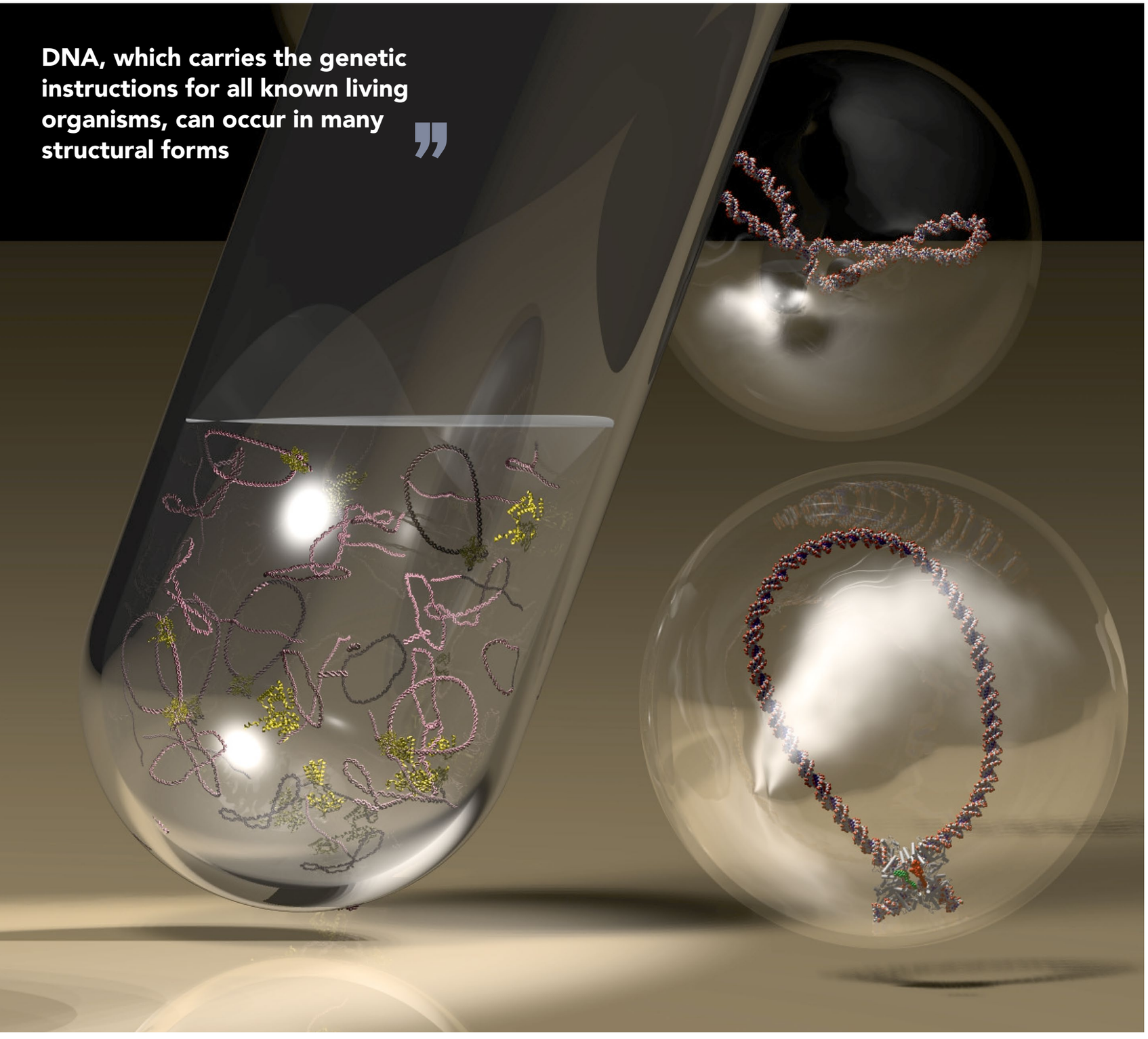


DNA, which carries the genetic instructions for all known living organisms, can occur in many structural forms

Single-molecule DNA topology and metagenomic circular DNA

Professor Stephen Levene investigates how the three dimensional architecture of DNA is involved in genome maintenance and gene expression. Based at the University of Texas at Dallas, the Levene lab uses sophisticated experimental and computational approaches to explore the flexibility and folding of DNA mediated by protein-DNA interactions. This work has led to valuable insights into the physics and organisation of genomes, the regulation of genes, and genetic recombination.

DNA, which carries the genetic instructions for all known living organisms, can occur in many structural forms: linear, circular, supercoiled (helically underwound or overwound), knotted or catenated (circular DNAs that are interlocked). The different forms of DNA can be distinguished by their topology – mathematical properties that are independent of the geometry of the molecule. Topology is defined as the properties of space that are preserved under continuous deformations, such as stretching or bending, but not tearing or gluing. Thus, short of breaking one or both DNA strands, the topology of a DNA molecule remains invariant even though its geometry may undergo large statistical fluctuations.

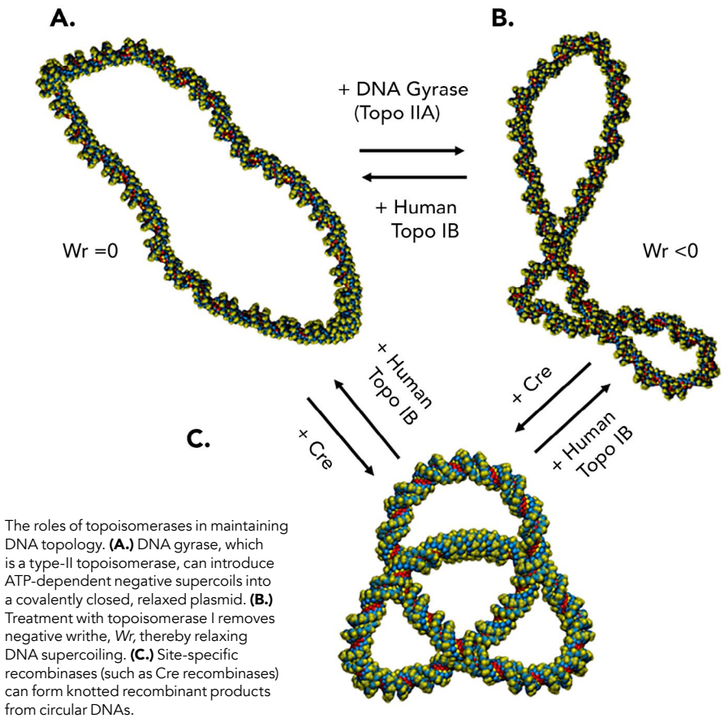
Co-discoverer of sequence-directed bending in kinetoplast DNA (circular DNA inside a mitochondrion), Professor Levene is motivated to better understand the connection between topological and geometric properties of DNA. By using a combination of experimental physical and computational approaches, the Levene lab aims to understand the roles of DNA topology in genome organisation, as well as the regulation of DNA organisation and topology by enzyme systems. The team employs an array of experimental methods including single-molecule imaging, bulk spectroscopic measurements, and novel and next-generation DNA sequencing methods, in combination with classical biophysical techniques such as gel electrophoresis and centrifugation. These experimental techniques are combined with complex computational models of DNA mechanics and protein-DNA structures, which are based on the physical principles of macromolecular behaviour.

WHEN BENCH-TOP SCIENCE AND COMPUTING BECOME ONE

Prof Levene and his group use mathematical methods to study the action of type-II topoisomerases on circular DNA. Type-II topoisomerases are enzymes that modulate the topology of DNA by breaking and re-joining DNA segments. Passing of duplex-DNA segments through enzyme-mediated transient DNA-strand breaks results in changes to DNA supercoiling and knotting that affects the distribution of topological states. The distribution of these states can be analysed using single molecule and ensemble fluorescence experiments (the latter involving large numbers of molecules), thereby allowing molecular interactions and conformational changes in solution and inside cells to be investigated. The use of these techniques in topological assays was made possible by a labelling technique developed in the Levene lab that can incorporate fluorescent dyes and other ▶

chemical modifications at multiple sites in circular DNAs.

The Levene lab has developed several methods and theories relating to DNA looping, which is strongly connected to both DNA topology and DNA geometry. One aspect of this work involved free-energy calculations for semi-flexible macromolecules, with a particular focus on DNA knotting and looping. Such calculations are challenging, especially for circular DNA molecules in the size range involving thousands of base pairs. This size range is particularly relevant when considering DNA-binding proteins that interact over typical distances in the genome, as occurs with gene regulation, DNA recombination and the action of type-II topoisomerases. The lab has also measured DNA-loop formation via Cre-mediated recombination, an enzyme system that is responsible for controlled genetic rearrangements in a bacteriophage (a virus that infects bacteria), but also has widespread use in genetic engineering. Because Cre recombination does not require additional accessory proteins or DNA supercoiling, it is a highly flexible system for quantitatively analysing DNA-loop formation both *in vivo* and *in vitro*.



The roles of topoisomerases in maintaining DNA topology. **(A.)** DNA gyrase, which is a type-II topoisomerase, can introduce ATP-dependent negative supercoils into a covalently closed, relaxed plasmid. **(B.)** Treatment with topoisomerase I removes negative writhe, *Wr*, thereby relaxing DNA supercoiling. **(C.)** Site-specific recombinases (such as Cre recombinases) can form knotted recombinant products from circular DNAs.

Alongside the groups' computationally based approaches, experimental techniques, such as agarose-gel electrophoresis are also used to optimise separation of DNA topoisomers (DNA molecules that are identical except for their topology) on the basis of knotting, catenation or supercoiling. The technique of gel electrophoresis is used to separate macromolecules based on their size and charge. An electric field is applied across a gel: more compact, negatively charged molecules migrate faster and move further through the gel toward the positive electrode because they can more easily migrate though the pores of the gel. Indeed, it has been shown that catenated, knotted, and supercoiled families of DNA behave differently in agarose-gel electrophoresis, depending on factors such as agarose concentration and the electric-field strength during electrophoresis.

Type-II topoisomerases play a critical biological role by maintaining the genome in an unknotted and uncatenated state; because these activities are essential to the survival of living cells, topoisomerases are major targets of cancer chemotherapy. However, destruction of cancer cells by these drugs is accompanied by unavoidable damage to healthy cells, leading to undesirable side effects and long-term health risks to patients who have undergone chemotherapy. Understanding more about topoisomerase mechanisms is necessary in order to improve the efficacy of new topoisomerase-targeted drugs and to minimise their side effects and risk of use.

METAGENOMIC CIRCULAR DNA

A small component of most genomes exists as independent circular DNA molecules. So-called extrachromosomal circular DNA (eccDNA) was first identified in plant cells in the 1980s, and has since been found in human cells and those of a number of other organisms. The biological role of these circular DNAs remains unclear; however evidence is emerging that eccDNA and the mechanisms that generate these molecules contribute via general and dedicated pathways to genome dynamics.

In addition to their work on DNA topology, the Levene lab aim to better understand the biological roles of eccDNAs; more specifically, their roles in genome instability and cancer. Increased levels of eccDNA have been observed in connection with developmental progression, ageing and genome stability. A subset of eccDNA elements has been associated with malignancies and drug-resistant tumours in a number of cancers.

However, it is only with the more recent development of high-throughput sequencing technologies that it has become possible to undertake extensive genomic mapping of eccDNA sequences. Prof Levene has collaborated with Dr Massa Shoura and Professor Andrew Fire at Stanford University to take a whole-genome approach to surveying the repertoire of eccDNAs in model organisms, e.g., *C. elegans*, as well as in normal and cancerous human cell lines. The team have characterised the 'circulome' (circular component of the genome) with an approach combining biophysical techniques that separate and characterise circular DNAs based on their topologies with next-generation sequencing methods. Their Circulome-Seq method uses newer capture and sequencing techniques, requiring smaller amounts of eccDNA, while avoiding methodologies that can selectively bias populations of eccDNA molecules. Their work showed that eccDNAs originated from a number of linear coding regions in the genome, as well as from repetitive non-coding elements, and that different cell types harbour different repertoires of circular DNAs. Although it is not yet clear what mechanism(s) drive production of circular DNAs from specific regions in specific cell types, insights into these processes may significantly impact our understanding of genome-maintenance activities and mechanisms that contribute to genetic diversity between cells. Moreover, continuing to investigate eccDNAs may allow differences between 'normal' and 'diseased' states to be identified. It is clear that there is still a lot to be learnt about how the three-dimensional structure of DNA, both in its linear-genomic context and as independent circular-DNA species, is involved in genome maintenance and gene expression, but the future looks exciting.

> **Continuing to investigate eccDNAs may allow differences between 'normal' and 'diseased' states to be identified**

Q&A

How did you first become interested in DNA structures?

I first became interested in DNA structures when I was around nine or ten years old – it was a topic presented in a grade-school science lesson. I've always been drawn to geometry problems both in mathematics and art. At that time I imagined the DNA bases to have different complementary visible colours, which they do not have in real life.

Technologies are advancing faster than ever, what is the next approach that you are excited to use to investigate DNA topology?

The time is right to think about investigating DNA topology at the level of single molecules. Not many techniques are yet available for doing this, but such methods may be important in characterising the topology of eccDNAs, which are present at pretty low levels in cells (about 1% of the genome's mass).

Do you think* in silico *methods will ever replace in-vitro/vivo methods?

I see *in-silico* methods as complementary to *in-vitro/vivo* experiments. One of the great strengths of this combination of empirical and computational approaches is that *in-silico* results can be invaluable in designing future experiments and fine-tuning models as well as analysing current data. Often *in-silico* calculations provide a kind of sanity check before undertaking a series of complex experiments, just to generate a prediction of what range of experimental outcomes can be anticipated. Nowadays many experiments are costly and time-/material-intensive – it's important to be sure that you have an idea of what results to expect.

We are seeing an increase in gene therapies; do you think eccDNAs could ever be used as one such treatment?

My collaborators and I are very interested in the possibility of using eccDNA analogs for biomimetic applications.

What cell lines or organisms would you like to target next with your Circulome-Seq method?

We are currently working on a large-scale survey of eccDNA in a wide range of normal and abnormal human cell lines and tissues. These surveys may guide us, with the help of CRISPR technology, toward investigating eccDNA profiles in engineered cell lines having targeted mutations.

Detail

RESEARCH OBJECTIVES

Research in the Levene lab applies chemistry, mathematics and molecular and cell biology expertise to challenges in biotechnology. Using sophisticated experimental and computational approaches the team explore the mechanics and dynamics of complex nucleoprotein structures, in order to elucidate the details of fundamental regulatory mechanisms.

FUNDING

- National Institutes of Health (NIH)
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- Cecil H. and Ida Green Foundation

COLLABORATORS

- Prof Andrew Fire, Stanford University
- Dr Massa Shoura, Stanford University
- Dr Andreas Hanke, University of Texas Rio Grande Valley
- Dr Walter Hu, University of Texas at Dallas

BIO

Prof Stephen Levene received his Ph.D. in Chemistry in 1985 from Yale University and was co-discoverer of the phenomenon of sequence-directed bending in kinetoplast DNA. Subsequently, he was an American Cancer Society postdoctoral fellow at University of California, San Diego in the laboratory of Bruno Zimm and a Staff Scientist in the Human Genome Center at Lawrence Berkeley Laboratory before joining the UT-Dallas faculty in 1992.

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