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 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.
quantitatively analysing DNA-loop formation and additional accessory proteins or DNA (a virus that infects bacteria), but also has a system that is responsible for controlled gene regulation, DNA recombination and distances in the genome, as occurs with DNA-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.

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 through 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 untreated 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.

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 such 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. One can also use these results to guide experiments, thus the two approaches are not mutually exclusive but can be used in tandem.

Continuing to investigate eccDNAs may allow differences between ‘normal’ and ‘diseased’ states to be identified.

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, while thereby relaxing DNA supercoiling. (C.) Site-specific recombinases such as Cre recombinase can form limited recombinant products from circular DNA.

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