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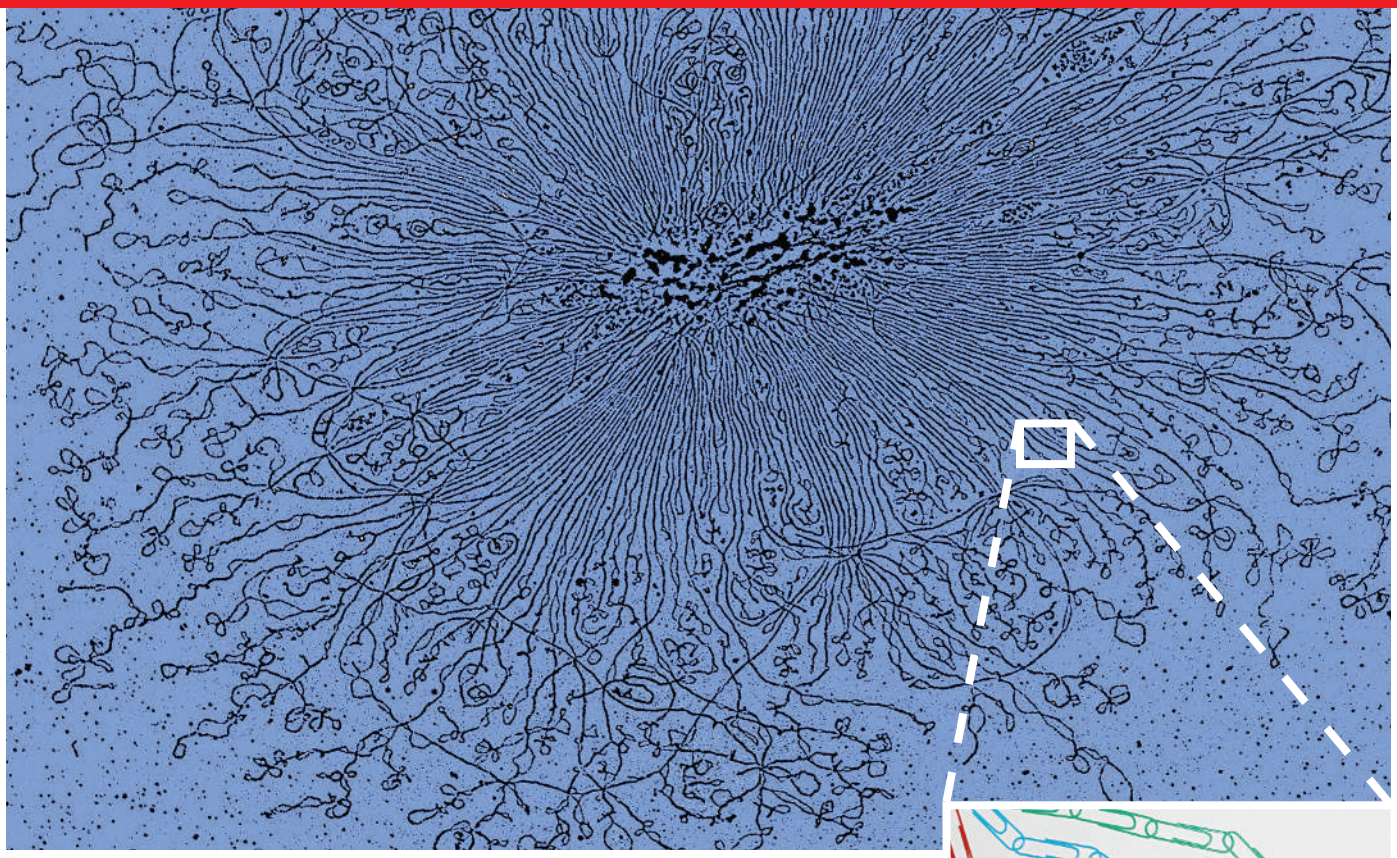


SPRING THEORY

Physicists interested in the mechanics of single molecules are helping open one of the blackest boxes in biology. **Brendan Maher** discovers how the disciplines are working together.

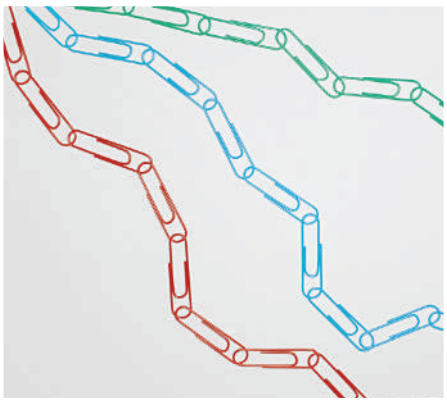
When trying to explain why he has become fascinated by physics lately, Kerry Bloom, a cell and molecular biologist at the University of North Carolina, Chapel Hill, pulls a handful of paper clips from his pocket and links them together. Stretching a small chain across the surface of his palm, he says: "Imagine this is DNA. You can stretch it to its full length, but each link in this chain is vibrating all the time." Bloom jiggles his hand, causing the paper clips to dance. The links twist at random, but once a couple of kinks or bends are introduced some force is needed to stretch the chain out again. After a few seconds of simulated brownian motion, the paper-clip chain collapses back into his hand.

Bloom's paper clips are a demonstration of the properties of an 'entropic spring', a system where thermodynamics favours a resting state in which all the chain's components are bunched and tangled. Rubber bands and silk share these properties, and so does DNA. It is not a new insight, but to Bloom, who has spent most of his career speaking the language of genetics, it's a powerful one. He gets a wistful look when talking about it — he's even written poetry about it ("Francis and Jim lasted 50 long years/Isn't it time for some big new ideas…"). Spring theory, as he calls it, might help explain a phenomenon he is deeply interested in — how the tiny biochemical machinery of the cell can manage billions of bits of information stored on vast polymer strings that need to be read, copied and packaged into an incredibly small space.

In the cartoon models that illustrate textbooks on cell- and molecular biology, purposeful proteins orchestrate neat, stepwise molecular dances as they react to coloured blobs and bind a perfect DNA staircase. Everyone finds their partner easily and does their job efficiently in a scale-free rendition of an otherwise empty space. The reality is something much more chaotic.

Eye of the storm

In the cell there's no eye-soothing white space to separate things. Water molecules are a constant omnidirectional hailstorm, van der Waals forces glue things together and viscosity rules. Within this molecular maelstrom, gravity is imperceptible, and there's more or less no inertia; all purposeful movement degenerates into random jittering the moment no further power is available. Bloom has a dramatic illustration of the strangeness: if a bacterium stops beating its flagella to move forward, it comes to a stop in "less than the width of a hydrogen bond", just a fraction of a nanometre.



Chains of life: DNA from a burst bacterium reveals a surprise at higher magnification.

For those who find these complications fascinating, the tools of modern physics are making them ever more amenable to study. Theoreticians and experimentalists are devising predictive mathematical models for the mechanical properties of cells at a molecular level, and starting to expose the formulae under which these tiny chaotic environments function. DNA, operating at the centre of this maelstrom, is of particular interest. "DNA is mechanically manhandled inside the cell," says John Marko, a condensed-matter physicist now working in Northwestern University's molecular-biology department in Evanston, Illinois — and that manhandling is important for replication, transcription, regulation, packaging and pretty much everything else DNA does or has done to it.

Oliver Rando, a biologist at the University of Massachusetts Medical School in Worcester who studies DNA packaging, hopes that physics may answer questions other approaches haven't touched. "You have these machines that appear all over the nucleus but only happen to

act at a couple of loci," he says, referring to specific places in the genome. "In some cases the detailed mechanism underlying that difference might be biophysical in nature." He's not certain that the physicists can solve the problem — but he's happy to see them try.

To find out how DNA works in the strange world of the cell, the first step is to look at how it behaves in simpler places. Carlos Bustamante, a pioneer in single-molecule biophysics at the University of California, Berkeley, got started in the field simply by thinking about the most everyday lab procedure: gel electrophoresis. DNA fragments loaded into a gel and then subjected to an electric field will migrate along the field lines, and the speed at which the different fragments do so reveals their size. In the late 1980s, when he was at the University of New Mexico, Albuquerque, Bustamante began to wonder about the details of the process, and used a microscope to watch fluorescently labelled DNA fragments migrating through a gel[1]. "What was amazing was how elastic they were," he says. As the electrical field pulled on the negatively charged strands, they folded and curled, crawling like caterpillars through the gel's molecular obstacle course of crosslinked polymers.

D. GOODSELL

Let's twist again

Bustamante started devising new experiments to stretch or twist the DNA and see how much force was needed to make the familiar double-helix structure break, unwind, or knot up like an old telephone cord. Key to these investigations were new and constantly improving ways of seeing and manipulating the molecular structures — by attaching beads of polystyrene to the ends or sides of long DNA molecules he could hold them in a magnetic field, or trap them with laser light and watch as the DNA squirmed and recoiled in reaction to what was done to it.

Because DNA is double-stranded and twisted, says Bustamante, it's quite rigid. But it also bends and folds — in fact it does so to an astonishing degree. The DNA in human cells is packed so tightly that two metres of it squeezes into every nucleus. The trade-off between rigidity and flexibility depends on scale: on small scales the molecule seems stiff, on larger ones bendy. Jonathan Widom, at Northwestern University, compares DNA to a garden hose; easy to wrap around your waist, impossible to wrap around your finger.

The key to the difference between stiff and flexible is the chain's 'persistence length' — the distance that, as Widom puts it, "defines how far you need to go along a polymer before it forgets which way it was going". For a strand of DNA left to itself, studies have pinned the persistence length at about 50 nanometres, which corresponds to 150 bases; below this length DNA is difficult to bend. Results from dozens of studies fit fairly well a pre-existing 'worm-like chain' model of DNA, which predicts that it behaves somewhat like a chain of tiny paperclips.

"If you knock out a gene and suddenly a rabbit doesn't like broccoli, that's not helpful to us."
— Philip Nelson

But key cellular processes, including packaging and genetic regulation, require looping on a scale much smaller than 50 nanometres. And some experiments by Widom and his collaborators showed tiny sequences spontaneously forming loops — 'cyclizing' — at a much higher frequency than would be predicted by the worm-like chain[2].

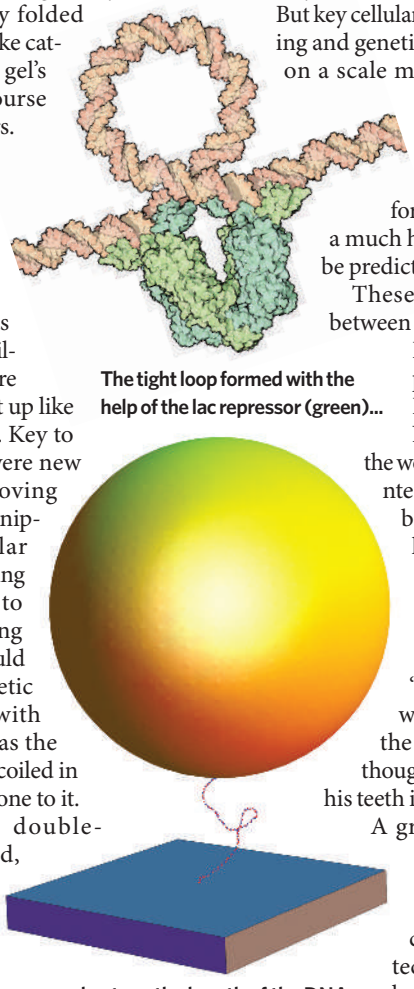
These are the sorts of mismatch between theory and reality that excite Philip Nelson, a theoretical physicist at the University of Pennsylvania, Philadelphia. Nelson says he took notice of the work by people such as Bustamante and others in the mid-1990s because it put biological problems into a language he could understand. "If you knock out a gene and suddenly a rabbit doesn't like broccoli," he says, speaking as a physicist, "that's not helpful to us." DNA wrapping itself up in knots that the models seemed to preclude, though, was a problem he could get his teeth into.

A group including Nelson and Widom recently approached the problem of tight looping using atomic-force microscopy, a powerful visualization technique that allows them to look at the shape of DNA strands directly, rather than looking at beads or fluorescence associated with them. They found that at lengths of between 5 and 10 nanometres (just 15 to 30 of the nucleotide subunits from which the double helix is built) the flexibility of the DNA was several orders of magnitude higher than that predicted by the worm-like-chain model. They proposed a new model called the sub-elastic chain[3]. Others, such as Marko and Jie Yan at the University of Illinois at Chicago had also been proposing models that allow for breakdown of the worm-like chain at short scales[4]. But it's still a contentious area in the field. "You have to say that this is very much in flux at the moment," says Widom, who notes that some of the assumptions in his cyclization experiments have come into question[5].

Widom has nonetheless found that he can make useful predictions of the bending and looping proclivities of a piece of DNA on the basis of its sequence. "That makes a link between bioinformatics and mechanics," he says. A DNA section with a specific nucleotide pattern might bend more or less than another strand, and some proteins seem to read this 'code' from DNA's bendability rather than directly from the sequence. Histones, the barrel-shaped packaging proteins around which DNA winds in tight curls are a prime example. According to Widom, the histones seem to prefer specific DNA sequences based on their flex. Rando, who works on histone dynamics, says this is where physics influences his work: "That's a case where something super-important to biologists is directed at least partially by something biophysics-y."

And histones aren't the only proteins known to manage DNA looping. The lac repressor, a tiny V-shaped protein that grabs two specific sequences of DNA about 10 nucleotides apart and pulls them together, forms a very tight coil in the intervening material — too tight for any proteins that might want to unspool and read the DNA to cope with. Jeff Gelles, a self-described "dyed in the wool *in vitro* biochemist" at Brandeis University in Waltham, Massachusetts, helped to develop a physical way to visualize this looping.

In 1991, he and his co-workers devised a simple way of looking at DNA mechanics visually[6]. They took a DNA strand that was being transcribed into RNA by a protein polymerase, fixed the polymerase to a glass slide and then attached a 40 nanometre gold bead to the free end of the DNA. The scale of the particle means that brownian motion has it dancing wildly — think balloon on a string in a hurricane — but the bead is big enough to see under a microscope. With time-lapse microscopy, the



K. TOWLES

The tight loop formed with the help of the lac repressor (green)...

...shortens the length of the DNA attached to this golden bead.

researchers could extrapolate the length and movement of the DNA from the bead's random positions around a central tether point.

If these tethered particle experiments can reveal DNA length, they can reveal DNA looping, as the looping, by taking a hitch out of the tether, shortens its effective length. In unpublished work, Oi Kwan Wong, a former graduate student with Gelles, now at Stanford University in Palo Alto, California, used tethered particles to investigate how much the looping caused by a lac repressor shortened a sequence of DNA that was equipped with the relevant binding sites. The only problem is that she saw three different lengths: one stretched out, unlooped length and two looped lengths. "We think that the most likely explanation for that is not something to do with the structure of the DNA, but rather that the repressor itself can undergo a large change in the three-dimensional structure," says Gellis.

Internal workings

Rather than using microscopic gold beads, Bloom is trying to study the dynamics of DNA inside the cell itself — using the cell's own machinery to do the work. During cell division a protein-motor complex called a spindle separates identical copies of each chromosome, pulling one towards one end of the cell and the other in the opposite direction, allowing the eventual daughter cells to each get a complete set. The spindle latches on to the chromosomes at structures called centromeres, which have been the focus of Bloom's work for decades.

Some years ago he engineered a cell chromosome with a second centromere that he could turn on and off. When the extra centromeres are activated during cell division, the spindle will sometimes latch on to two centromeres on the same copy of a chromosome, stretching it across the cell rather than separating it from its twin. Occasionally, the stretched chromosome snaps like a wishbone, with the DNA recoiling to one end of the dividing cell.

Bloom originally used the method to study how the cell responds to DNA breakage[7]. But he is now starting to look at the dynamics of the break itself, using a laser to snip the stretched chromosomes and measuring the rate at which they recoil. "This is now where it gets complicated, and I'm not an expert," says Bloom. "We can see it stretch, we can see it recoil. How do I deduce force?" Elements of the worm-like-chain model predict his observations fairly well, he says, and with additional genetic manipulation, he can begin to look at how histones disassemble and reassemble in the stretched and recoiling DNA.

The moxie required to study DNA physics inside the cell itself elicits both admiration and scepticism. "I would say that it is really important to understand how these models work inside the cell," says Bustamente. "But let's not forget that the best biophysics is always done outside the cell." Hermann Gaub, a professor at Ludwig-Maximilians University in Munich, Germany, says he thinks observing systems that include some of the DNA's biological setting, but that stay away from the messiness of the cell proper are likely to be the most fruitful: approaches such as that of Marko, who pokes, prods and pulls the peculiarly large chromosomes of newts. "Doing it right inside the cell is what I would call heroic," says Gaub. But he's nonetheless intrigued.

Bloom admits that the work is preliminary. He says he's been boning up on polymer theory and is probing for potential collaborators — but he wants the data to show them first. "This is the stuff physics brings to the table," he says. The packaging of all that DNA into the nucleus of cell, he continues, "is another one of these big mysteries". A physical sense of how a hierarchy of folding patterns can pack DNA into the cell but also allow its sequences to be accessed remains far off. "By most estimations, it's packaged 10,000 fold," says Bloom, and no one knows how. "That's the attraction."

It's far from the only problem that physicists have their eyes on. "People are now able to do extremely quantitative and extremely reproducible and precise experiments on individual biomolecules," says Marko, "and that's very attractive to physics people." And there are many biomolecules to choose from. To biologists, a text such as *Molecular Biology of the Cell* by Bruce Alberts and his colleagues is a well-trodden rendition of that which is known. But to a physicist first approaching biomolecules, it's an Aladdin's cave of shiny and captivating phenomena. Nelson says he's drawn to the sensation when something in biology "makes you ask 'How the heck does that happen?' Physicists start reading Alberts' and they say that every three pages." ■

B. NALLEY/S. WHITFIELD

Resting state: paper clips and a touch of photoshopped DNA make their point in the palm of Kerry Bloom.

Brendan Maher is a features editor at *Nature*.

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