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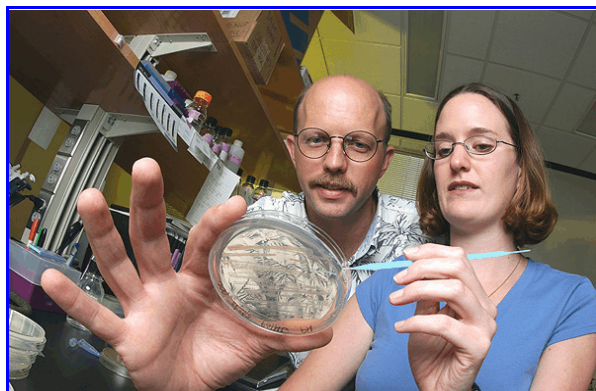
HOLD THE SALT, PLEASE: LOWERING SALT CONTENT IN DNA SOLUTIONS MAY HELP IMPROVE GENE THERAPY SUCCESS

Researchers have found they can control the size of densely packed DNA structures by changing the salt concentration in solutions containing DNA. The finding could improve the efficiency of gene delivery for medical treatment and disease prevention.

Scientists are seeking to understand the natural mechanism of DNA condensation into nanostructures -- in particular, toroids, which look like tightly wound garden hoses. Densely packed DNA is nature's efficient way of transporting genetic information, done particularly well by sperm cells and viruses.

Researchers want to mimic this process to improve DNA delivery for gene therapy and DNA-based vaccines, but they face many challenges in the laboratory where DNA in solution typically exists in an extended, rather than condensed state. Scientists have been able to cause DNA to condense into toroids by adding positively charged molecules to samples, but they have had difficulty finding the right molecules to achieve consistent, optimal toroid sizes of less than 50 nanometers.

However, scientists at Georgia Institute of Technology have made a significant advance in controlling the size of DNA toroids. In the July



Associate Professor Nicholas Hud (left) and Ph.D. students Christine Conwell and Igor Vilfan (not shown) have made a significant advance in controlling the size of DNA toroids.

18, 2003 online issue of the journal *Proceedings of the National Academy of Sciences* (PNAS), they report that reducing salt concentrations below normal laboratory solution levels shrinks both the diameter and thickness of DNA toroids. This finding resulted from a combined investigation of how static DNA loops and solution conditions might be used to control toroid dimensions.

"But even without static loops present, DNA produces smaller toroids if you reduce the salt concentration," said Nicholas Hud, an associate professor of biochemistry who is

leading the study funded by the National Institutes of Health. "We found a systematic relationship between reducing salt and reducing toroid size. It is surprising that such a study was not previously done because salt concentration is such a fundamental parameter in studying molecules in solution, particularly such highly charged molecules as DNA."

Protocols for preparing DNA for delivery to cells often call for salt conditions that differ from those DNA encounters when injected into body tissues, Hud noted. "If you change the salt conditions during DNA delivery, it will change particle size and have a dramatic effect on the efficiency of gene delivery," he added. "This could explain why some researchers aren't getting as good a rate of transfection (the incorporation of DNA into a cell) as they should."

In the study reported in PNAS, Hud and his Ph.D. students Christine Conwell and Igor Vilfan also describe using the positively charged, inorganic molecule hexamine cobalt (III) to condense a DNA molecule containing a specially designed sequence. The synthetic sequence causes a region of the DNA molecule to bend into two loops of 25 nanometers each in diameter. In other words, these nanoscale loops were "programmed" into the DNA sequence.

Hud theorized years ago that the spontaneous formation of loops along DNA is the first step necessary for toroid formation, and a key factor in determining toroid size, he said.

"Now, we've made these loops always present there," Hud explained. "When we add positively charged molecules that bind to the DNA, the loops provide a built-in starting point for DNA condensation. The loops also act as a template upon which the rest of the DNA rolls up to form a toroid. The toroid forms because the positively charged molecules make DNA want to stick to itself.... and we found that our static loops reduce DNA particle size and tighten particle distribution."

Hud describes as serendipitous the additional finding that lowering the salt concentration in DNA solution also reduces the size of toroids. Together, these results helped Hud's team develop models for DNA toroid

formation. The researchers' data can now serve as a test for theoretical models of DNA condensation, they say.

Meanwhile, Hud's team is exploring how the order in which they add salts to DNA solutions affects particle size and shape -- whether salts should be added before or after the DNA condensation process is prompted by positively charged molecules.

"By studying the fundamental process of DNA condensation we hope to determine all the factors that help produce particles of smaller size and narrower size distribution. The combined effects of these factors should help us to produce the optimal particles for gene delivery," Hud added.

He believes a systematic approach to the high-stakes goal of developing an efficient, artificial gene delivery method will pay off.

"There have been a lot of attempts to improve DNA delivery by simply mixing molecules and empirically testing to determine their efficiency," Hud said. "But DNA condensates are difficult to understand.... We might be missing something in our information about what's happening from the lab bench to the delivery of DNA to cells. We want to understand the nature of DNA particles all the way from the test tube to the cell."

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