PHYSICISTS PUZZLE OVER GEL ELECTROPHORESIS OF DNA

The human genome project aims to determine the sequence in which the four bases of DNA occur in each of the 24 different chromosomes in the nucleus of a human cell. Chemical staining techniques indicate that the human chromosomes are all discernibly different in size, and contain between 50 million and 250 million base pairs. But present-day techniques cannot give more definitive numbers for the sizes of the human chromosomes, in part because they are the largest well-defined molecules modern science has encountered and weight standards and analytical techniques for handling such large molecules are not very well developed.

Molecular biologists would like very much to be able to separate the 22 autosomal and 2 sex chromosomes in a human cell and even accumulate large numbers—on the order of, say, 10^9 molecules—of each species. The present-day techniques for obtaining the sequence of base pairs in a chromosome are biochemical and work with much smaller DNA segments than occur in whole chromosomes. If a chromosome is broken up into small fragments each of which is sequenced separately, one must determine the order of the fragments to obtain the base-pair sequence for the entire chromosome. This is much easier to do when one knows for sure that all the fragments that one sequenced came from the *same* chromosome.

Clearly, since the chromosomes all consist of DNA, a solution or collection of a given species of chromosomes differs from that of another species most visibly in those physical properties that depend on the chromosomes' molecular weight. And it is these properties that any separation technique must exploit. (The number of base pairs in a DNA molecule is a more interesting and useful measure of its size than the molecular weight. A base pair weighs about 660 atomic mass units, or daltons.)

Molecular biologists have traditionally used electrophoresis for separating large molecules, such as proteins and DNA fragments, according to size. In what may now be called the standard version of this technique for separating DNA molecules, the molecules diffuse through the pores of a gel under the influence of a uniform electric field that is also constant in time. This so-called constant-field gel electrophoresis can separate DNA molecules of sizes up to 50 kilo-base pairs. The resolution is better than 10%, so that molecules of 40 and, say, 42.5 kilo-base pairs can be separated. In the mid-1970s, however, molecular biologists discovered to their dismay that electrophoresis of DNA in an agarose gel, the medium of choice for DNA, did not resolve very well DNA fragments larger than 50 kilo-base pairs. Attempts to overcome this barrier by, for example, diluting the gel to increase the size of the pores, through which the molecules move as they diffuse under the influence of the electric field, were not very successful.

Pulsed-field electrophoresis

Separation of DNA molecules containing up to 2 million base pairs (mbp), which is routinely carried out in laboratories around the world these days, suddenly became possible in 1983. That year David Schwartz and Charles Cantor at Columbia University succeeded in separating intact the 16 chromosomes of yeast, which have sizes between 230 and 2500 kbp, using a new type of gel electrophoresis in which the electric field was pulsed between the two orthogonal directions in the plane of the gel and the field in at least one of the two directions was nonuniform.[1]

The discovery of pulsed-field electrophoresis is one of the advances that "made the human genome project possible," according to Cantor, who now heads DOE's Human Genome Center at the University of California, Berkeley. The story of this remarkable discovery is the stuff that scientific dreams are made of: Schwartz, who was Cantor's graduate student at Columbia when the two published the seminal paper on pulsed-field electrophoresis, had several years earlier come up with the basic idea of pulsing the electric field to increase the size range of DNA fragments that could be separated using electrophoresis. He had even done some preliminary experiments in Bruno Zimm's laboratory in 1979–80, while he was a beginning graduate student in chemistry at the University of California, San Diego. He is now in the chemistry department at New York University.

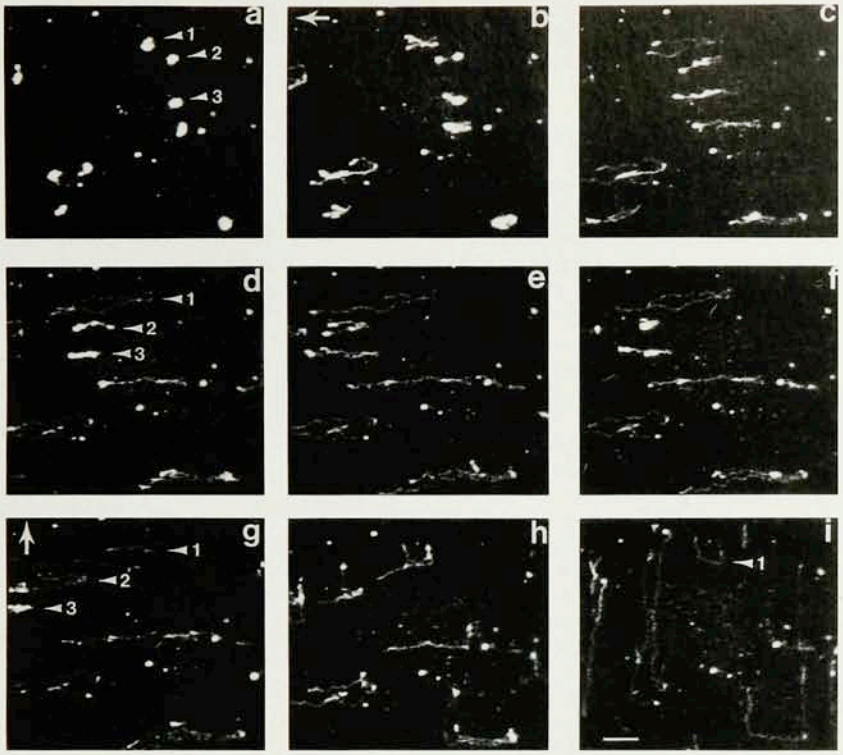
The inspiration for pulsing the electric field to overcome the barrier to separation of molecules in constant-field electrophoresis came, Schwartz told us, from the well-known viscoelastic properties of solutions of long polymers. One interesting manifestation of these properties occurs when the space between two concentric cylinders is filled with a polymer solution and the inner cylinder is rotated while the outer one is held stationary. In response to the shear force acting on them due to the inner cylinder's rotation, the polymers uncoil and align themselves along the streamlines. When the generator supplying the torque to the inner cylinder is turned off, the cylinder begins to rotate in the opposite direction as the polymers coil back into their equilibrium conformations. The time constant for the polymers' return to equilibrium, and therefore for the decay of the inner cylinder's reverse rotation, depends on the molecular weight of the polymer. This dependence is useful for measuring the size of polymers but not for separating polymers according to size.

DNA molecules containing several thousand base pairs have linear sizes comparable to the cross section of the pores in the gel. The pores in agarose at 1% concentration in water are typically three-tenths of a micron across. Might not certain conformations of these molecules, Schwartz reasoned, such as those that aligned the molecules with the pores, be more suited to their motion through the pores? Furthermore, he argued, since the viscoelastic effects show that the time constant for the polymers to change conformations depends on the molecular weight, separation of molecules by weight should be possible if one could force such a conformational change. This is where the idea of pulsing the electric field came in.

One of the crucial parameters for the success of the first experiment, Schwartz told us, was the period of the field pulses. DNA molecules containing several hundred thousand base pairs are quite sluggish. In their first experiment, Schwartz and Cantor used pulses of 1–90 seconds' duration.

Polymer science

Some of the advances in theoretical polymer science in the past two decades have centered around the concept of a hypothetical tube in which each polymer in, say, a dense solution of polymers may be regarded as being confined. Polymers in such solutions may fluctuate only between those conformations in which no part of them extrudes from the hypothetical tube. Sam Edwards of the University of Cambridge proposed this concept in



DNA molecules undergoing electrophoresis. DNA molecules from the G bacteriophage, which contain 700 kilo-base pairs, were stained with a dye and observed using a fluorescence light microscope. Under the application of a uniform electric field of 3.5 V/cm, molecules 1, 2 and 3 in panels **a**–**f** elongate and move in the field direction (arrow in **b**). The molecules reorient (panels **g**–**i**) when the field is rotated by 90° (to point in the direction of the arrow in **g**). The continuing movement produces a series of hook structures and thick rod-like coil conformations. The time interval between successive panels varies, and ranges from 5 sec to 30 sec. The bar in **i** stands for 10 microns. (From ref. 6.)

1967 to explain the elasticity of rubber. In 1971 Pierre-Gilles de Gennes considered the wriggling motion of the confined polymer, which leads to a shift in the polymer's center of mass, causing diffusion. De Gennes called this motion reptation, after the Latin verb *reptare*, meaning "to creep."

At the end of the 1970s, when the details of the reptation theory were worked out, many polymer theorists would have thought that reptation also described the dynamics of polymers undergoing electrophoresis. DNA is a uniformly charged polymer in water or other neutral environments, because each phosphate group in the polymer's helical backbones gives off a hydrogen ion (which is why the solution behaves like an acid). The polymer therefore has a uniform negative charge along its length. Simple theoretical analyses of the dynamics of such polymers that regard reptation as the primary mode for the polymers' motion in the constraining pores of the gel predict an inverse relationship between the polymer's mobility and its mass.[2] This explains the size resolution obtained in standard, constant-field electrophoresis.

Of snakes and inchworms

In 1987 Jaan Noolandi and his collaborators at the Xerox Research Center of Canada at Mississauga and at the Biotechnology Research Center at Montreal carefully examined electrophoresis in a constant field using a modified reptation model. The modification lay in the assumption that either end of the polymer could come out of its enclosing tube in the presence of an electric field, because both ends of the molecule carry the same charge. The modified model reproduced the barrier to resolution in a uniform and constant field that the molecular biologists had discovered in the mid-1970s and that theoretical analyses two years earlier had confirmed, but it also yielded the unexpected prediction that there would be an inversion of the size–mobility relationship at large sizes: Analyses based on this so-called biased-reptation model predict that the relationship between the mobility and size is not monotonic, but rather that the decrease in mobility with increasing size saturates at some critical size whose value may depend on the electric field, so that the mobility actually increases with size for molecules larger than the critical size.[3] In their 1987 paper, Noolandi and his collaborators also showed experimental data in support of their prediction of the inversion in the mobility–size relationship, but the enormous increase in the range of DNA sizes that could be separated by using time-dependent electric fields lay unexplained.

Also in 1987, Joshua Deutsch (University of California, Santa Cruz), following up on his earlier collaborative work with Edwards and Monica Olvera de la Cruz (Northwestern University) on polymers in gels, started simulating the problem numerically using a simple model.[4] The simulations by Deutsch, and later ones by Deutsch and Thomas Madden (also of Santa Cruz), showed that polymers undergoing electrophoresis move not at all like "snakes through tall reeds," but rather like "inchworms." Deutsch found that a polymer undergoing electrophoresis may form a U-shaped kink when it gets entangled on an obstacle in the gel and that the chain can leak out of this configuration with *either* end leading. With entangling on and subsequently sliding over obstacles as the primary mode of motion, a polymer under pulsed fields alternates between extended and coiled configurations, somewhat like an inchworm, which moves by "alternately bunching up and extending."[4] Simulations by Olvera de la Cruz, Dilip Gersappe and Edward O. Shaffer (all from Northwestern University) in which they also calculate the distance between the ends of the polymer show that the alternation between extended and coiled conformations may occur even in constant electric fields.[5]

Experiments have confirmed the new dynamics of DNA revealed in numerical simulations of electrophoresis. The figure on this page, obtained by Schwartz and Michael Koval at the Carnegie Institution of Washington last year, show individual DNA molecules stained with a fluorescent dye and photographed using a microscope.[6] The first six panels in the figure show the molecules migrating in a constant electric field

(which points in the direction of the arrow in panel b). Clearly the molecules, which are coiled up in random conformations initially (panel a), get elongated and align in the direction of the field. The molecules also get wrapped around obstacles in the gel (molecule 1 in panel c), and the tension between the two arms of the "hook" that is thereby formed is relieved when one arm becomes dominant and pulls the other arm past the obstacle (panels d–f). All the molecules begin to reorient themselves when the field direction is changed, as in panels g–i, which were obtained after the field direction was switched by 90°, so that it pointed in the direction of the arrow in panel g. Similar pictures have been obtained at the University of Washington, Seattle, by Steven B. Smith, P. K. Aldridge and J. B. Callis and at the University of New Mexico by Carlos Bustamaente and his collaborators.

The usefulness of theoretical concepts such as reptation lies in the simple explanations they offer for complex phenomena. Using the reptation model it takes a few lines to derive, for example, that the response of a dense collection of polymers to external perturbations will be different at long and short times and that the characteristic time scale for the onset of the long-time behavior will vary as the third power of the molecular weight. (See the article by de Gennes in PHYSICS TODAY, June 1983, page 33.) Experiments find a value close to 3.3 for the exponent giving the dependence of the characteristic time scale on the molecular weight.

Theorists therefore continue to ask whether the models of polymer dynamics based on the notion of the enclosing tube or of reptation are inapplicable to gel electrophoresis. Several physicists have extended those notions to explain polymer mobilities in response to pulsed fields. For example, Zimm has considered a model in which large amounts of the polymer may accumulate in wide pores that are connected by narrow ducts. Noolandi and his collaborators and, independently, Thomas Duke of the University of Cambridge have adapted the biased-reptation model to allow for fluctuations in the length of the enclosing tube.[7] Many of these physicist claim that their modifications of the reptation model can explain some feature of pulsed-field electrophoresis, such as the minimum in the mobility of polymers of a given size as a function of the pulse duration. This mobility minimum, which also appears in Deutsch's model, occurs when the pulse durations are comparable to the response time of the molecules, so that the molecules merely change conformations in each pulse cycle and do not have time to diffuse. Schwartz and Cantor were aware of this possibility and therefore cautioned in their very first paper that the choice of proper pulse periods was crucial to the success of the separation they had achieved.

What, then, have all the theoretical models achieved? Molecular biologists are quite enthusiastic about the insights into the dynamics of DNA that the theoretical investigations have revealed. A variety of new procedures for pulsing the electric fields used in electrophoresis have been developed since the paper by Schwartz and Cantor, including one in which the field is reversed but, to obtain net mobility, the duration of the pulse in one direction is longer than that in the reverse direction and the magnitudes of the fields in the two directions are also different. Separating DNA molecules in a given size range using pulsed-field gel electrophoresis has now become a complex optimization problem in which the field magnitudes, the field directions and the field durations are variables whose values must be carefully adjusted. The best protocols at the genome center at Berkeley, Cantor told us, can resolve molecules up to 5–5.5 mbp long with better than 10% resolution. To achieve good separation in that size range may require running times of up to 120 hours.

Computer codes based on the biased-reptation model, according to Noolandi, are very effective in working out the most efficient protocol for pulsing the electric fields. He said that protocols in which the electric field is quasiperiodic with as many as 30 characteristic periods can separate molecules in the 10–20-mbp range.

Several experimenters, such as George Holzwarth (Wake Forest University), Bjorn Åkerman and Bengt Norden (Chalmers University of Technology, Göteborg, Sweden), and John A. Schellman (University of Oregon) are studying the factors that determine the response time of DNA molecules by measuring the acceleration and alignment of DNA during electrophoresis.

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