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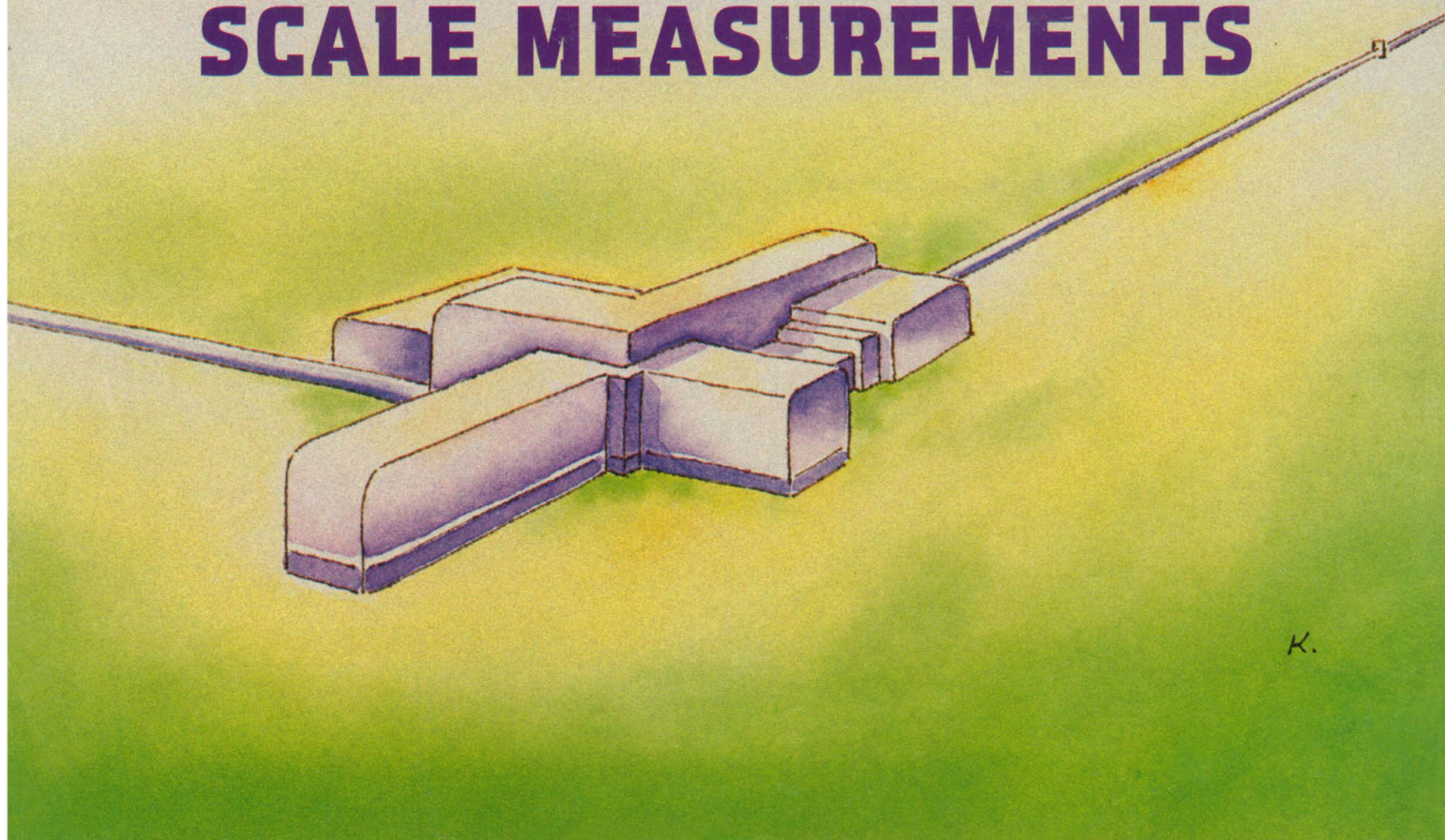
# SCIENCE

17 APRIL 1992

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VOL. 256 • PAGES 281-412

## LARGE SCALE MEASUREMENTS



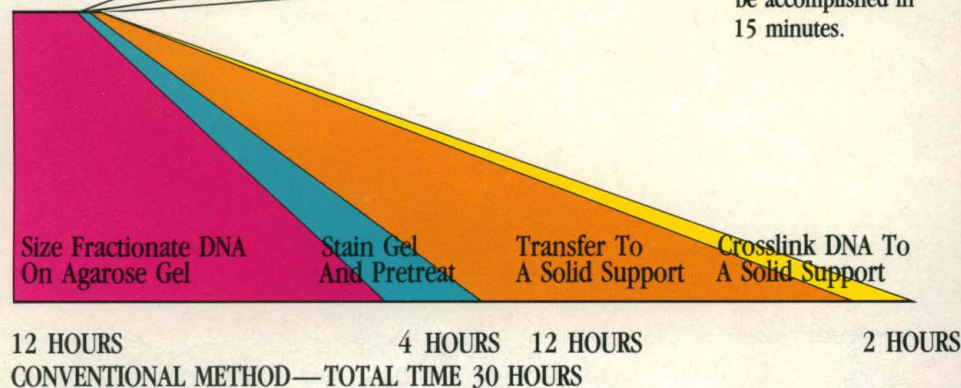
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# Southerns/Northerns: Electrophoresis, Blotting, and Crosslinking in 2.5 Hours Instead of 30.

Stratagene has streamlined agarose gel electrophoresis and blotting. The system decreases the time required, from sample loading to prehybridization ten fold.

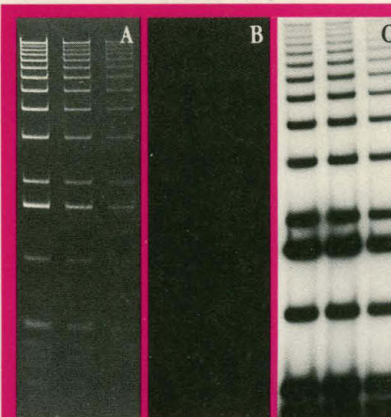
STRATAGENE METHOD—TIME 2.5 HOURS  
2 HOURS 15 MIN 15 MIN 30 SECONDS



## VAGE™ System

The VAGE™ vertical agarose/acrylamide gel electrophoresis system allows the casting of agarose or acrylamide gels in the unit. Nucleic acids can be electrophoresed through a 3 mm, 0.8% vertical agarose gel in less than two hours with excellent resolution (Figure 1).

Because the gels are thin, staining, depurination, and denaturation can be accomplished in 15 minutes.



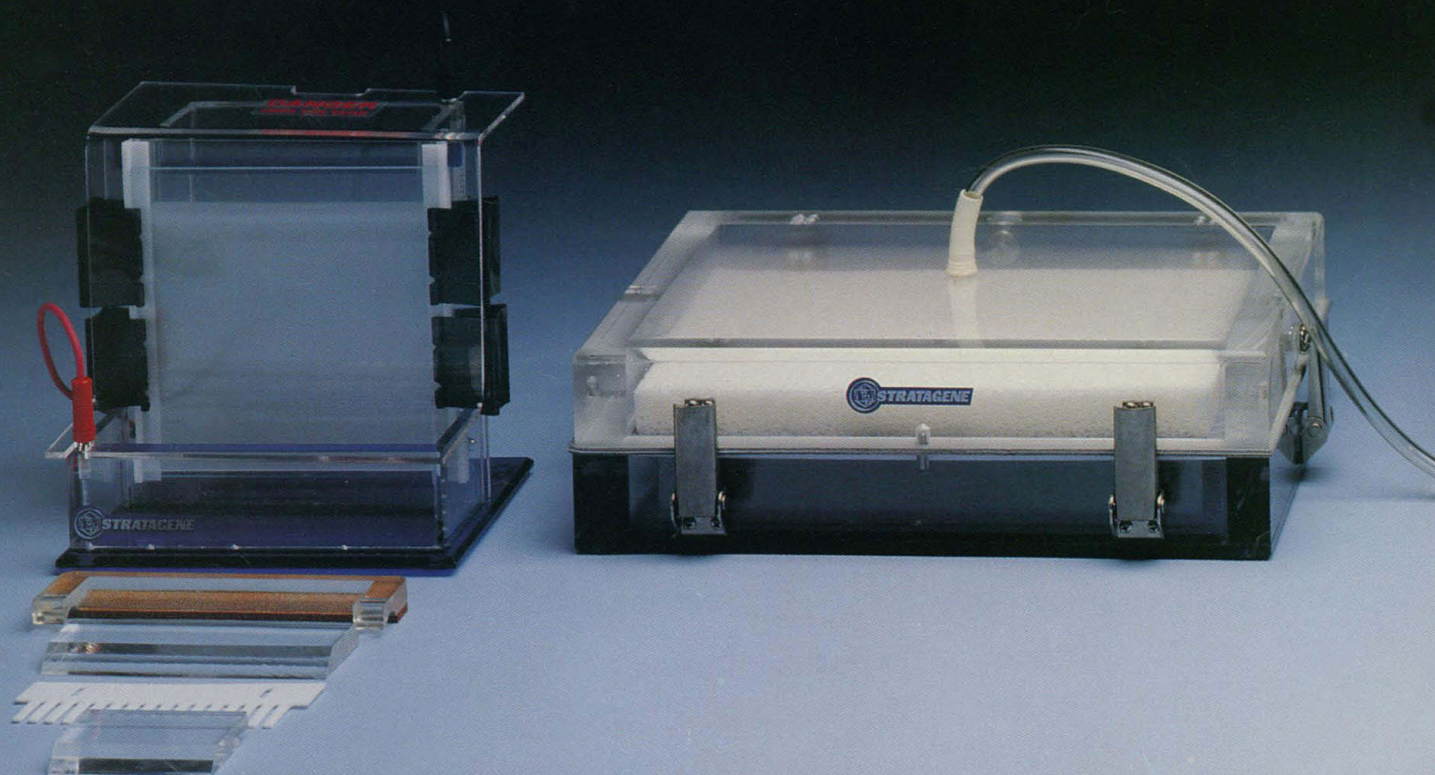
**FIGURE 1:**

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.

A. Ethidium stained gel showing high resolution.

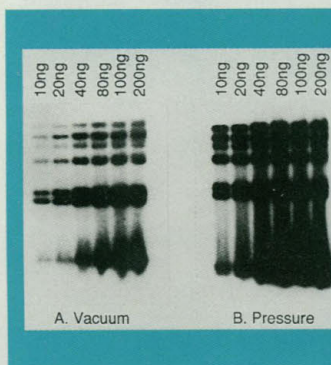
B. Same gel after pressure blotting.

C. Autoradiogram of membrane after pressure transfer.





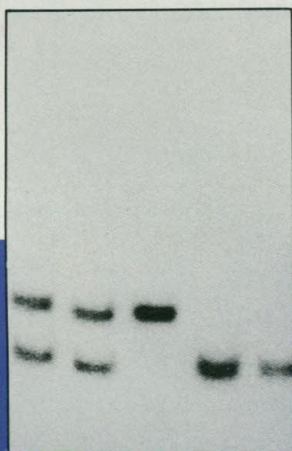
## PosiBlot™ Pressure Blotter



**FIGURE 2:**

Figure Legend:  $^{32}\text{P}$  end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

The PosiBlot™ positive pressure blotter permits the transfer of nucleic acids in 1/3 the time of vacuum blotters and 1/50 the time of capillary blotting (Figure 2). Pressure blotting does not dehydrate gels as do other methods. This allows the use of substantially higher pressure differentials, compared with vacuum blotting, without gel collapse. The PosiBlot apparatus reduces blotting time to 15 minutes.

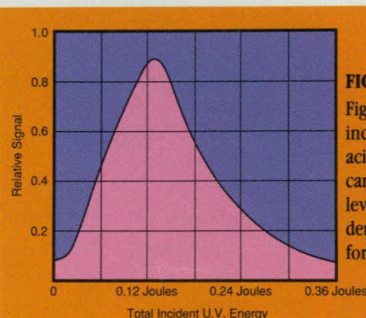


**FIGURE 3:**

Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

## Stratalinker™ UV Crosslinker

The Stratalinker™ UV Crosslinker fixes nucleic acids to solid supports such as nitrocellulose or nylon membranes, in less than one minute. This compares favorably to vacuum baking, which requires 2 hours. The Stratalinker actually monitors the ultra violet energy flux and deactivates the light source upon reaching the user-programmed energy level (Figure 4). Figure 3 shows an autoradiogram of a human genomic Southern blot performed using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.



**FIGURE 4:**

Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

Stratagene offers a full selection of nitrocellulose, reinforced nitrocellulose and nylon membranes. Each membrane is stringently lot tested to ensure consistency when performing Northern and Southern blotting. Please call Technical Services for detailed information on Stratagene's time saving blotting systems and membranes.



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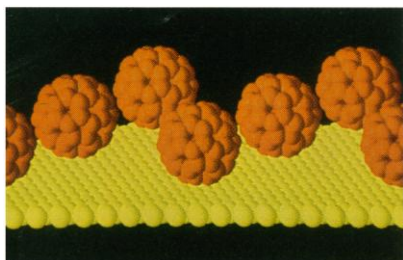
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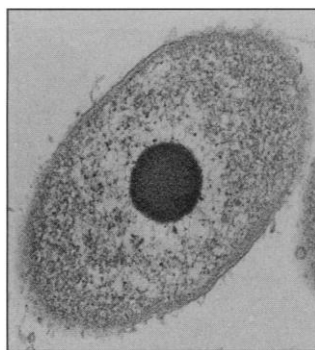
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Chemistry in *computero* **306**



**377** Bacterial nucleoid  
condensation

## NEWS & COMMENT

- DNA Fingerprinting: Academy Reports **300**
- Why Watson Quit as Project Head **301**
- Was Argonne Whistleblower Really  
Blowing Smoke? **303**
- Kinsey Institute Director Sues  
Indiana University **304**
- New Clinical Trial Planned **305**
- Physics Facilities Come Under Fire:  
DOE Bites the Bullet • Whacking at the SSC **305**

## RESEARCH NEWS

- The Ascent of Odorless Chemistry **306**
- Anthropologists Bet on Their Latest  
Data in Las Vegas **308**
- New Methods Make Mid-Sized  
Molecules Easier to Solve **309**
- Chemists Vie to Make a Better Taxol **311**
- Quasars: Ablaze With Gamma Rays **311**

## SPECIAL SECTION

### Large Scale Measurements

#### NEWS REPORTS

- Turning a Keen Eye on the Stars • A **316**  
Military Navigation System Might Probe  
Lofty "Weather" • Cosmologists Search the  
Universe For a Dubious Panacea

#### ARTICLES

- The Hubble Constant **321**  
J. P. Huchra
- LIGO: The Laser Interferometer **325**  
Gravitational-Wave Observatory  
A. Abramovici, W. E. Althouse, R. W. P. Drever,  
Y. Gürsel, S. Kawamura, F. J. Raab, D. Shoemaker,  
L. Sievers, R. E. Spero, K. S. Thorne, R. E. Vogt,  
R. Weiss, S. E. Whitcomb, M. E. Zucker
- Global Tectonics and Space Geodesy **333**  
R. G. Gordon and S. Stein
- Measured Trends in Stratospheric Ozone **342**  
R. Stolarski, R. Bojkov, L. Bishop,  
C. Zerefos, J. Staehelin, J. Zawodny

## DEPARTMENTS

- THIS WEEK IN *SCIENCE* **287**
- EDITORIAL **289**
- LETTERS **292**  
The Insanity Defense and Mental Illness: W. T.  
Carpenter and J. R. Rapoport; M. Sabshin, H. A.  
Pincus, W. Davis; P. R. Marques; B. J. Ballermann;  
D. E. Koshland, Jr. • Effects of Low Levels of Lead  
Exposure: J. F. Rosen; H. L. Needleman
- SCIENTESCOPE **299**  
Epidemiologists head down to the farm; etc.

- RANDOM SAMPLES **312**  
Saving Forests With Their Own Medicine • A 100%  
Certifiable Massive Black Hole? • \$10 Million for  
Ukraine Scientists • El Niño Still on the Prowl •  
Thousands of Insects 'Enroll' at Yale; etc.
- BOOK REVIEWS **388**  
The Los Alamos Primer, reviewed by F. J. Dyson •  
The Seeds of Time, A. M. Silverstein • Some Other  
Books of Interest • Vignettes • Books Received
- PRODUCTS & MATERIALS **391**

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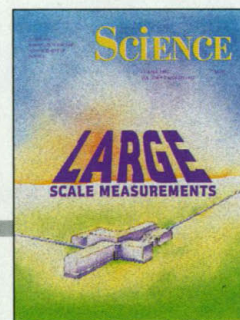
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## COVER

Illustration of one site in the proposed Laser Interferometer Gravitational-Wave Observatory (LIGO). The LIGO facilities will consist of two such interferometers located at widely dispersed sites; scientists hope LIGO will be able to detect gravitational waves emanating

from collisions of black holes and neutron stars. See page 325. A special section in this issue of *Science* focuses on large scale measurements; see the Editorial and pages 316 to 349. [Illustration: Ruth Sofair Ketler]



## RESEARCH ARTICLE

- Allosteric Effects of Nucleotide Cofactors on *Escherichia coli* Rep Helicase-DNA Binding** 350  
I. Wong and T. M. Lohman

## REPORTS

- $^{40}\text{Ar}/^{39}\text{Ar}$  Dating of the Brunhes-Matuyama Geomagnetic Field Reversal** 356  
A. K. Baksi, V. Hsu, M. O. McWilliams, E. Farrar

- Recognition of Paleobiochemicals by a Combined Molecular Sulfur and Isotope Geochemical Approach** 358  
M. E. L. Kohnen, S. Schouten, J. S. S. Damsté, J. W. de Leeuw, D. A. Merritt, J. M. Hayes

- Contact Electrification and Adhesion Between Dissimilar Materials** 362  
R. G. Horn and D. T. Smith

- An Efficient Antibody-Catalyzed Aminoacylation Reaction** 365  
J. R. Jacobsen, J. R. Prudent, L. Kochersperger, S. Yonkovich, P. G. Schultz

- Identification of a Naturally Occurring Transforming Variant of the p65 Subunit of NF- $\kappa$ B** 367  
R. Narayanan, J. F. Klement, S. M. Ruben, K. A. Higgins, C. A. Rosen

- Calcium-Regulated Phosphorylation Within the Leucine Zipper of C/EBP $\beta$**  370  
M. Wegner, Z. Cao, M. G. Rosenfeld

- Alternative Forms of Max as Enhancers or Suppressors of Myc-Ras Cotransformation** 373  
T. P. Mäkelä, P. J. Koskinen, I. Väström, K. Alitalo

- Nucleoid Condensation in *Escherichia coli* That Express a Chlamydial Histone Homolog** 377  
C. E. Barry III, S. F. Hayes, T. Hackstadt

- Reciprocal Regulation of Adipogenesis by Myc and C/EBP $\alpha$**  379  
S. O. Freytag and T. J. Geddes

- Cell Cycle-Regulated Binding of c-Abl Tyrosine Kinase to DNA** 382  
E. T. Kipreos and J. Y. J. Wang

- Appearance of Water Channels in *Xenopus* Oocytes Expressing Red Cell CHIP28 Protein** 385  
G. M. Preston, T. P. Carroll, W. B. Guggino, P. Agre



**333** Wide regions of deformation associated with plate boundaries

■ Indicates accompanying News story or Perspective

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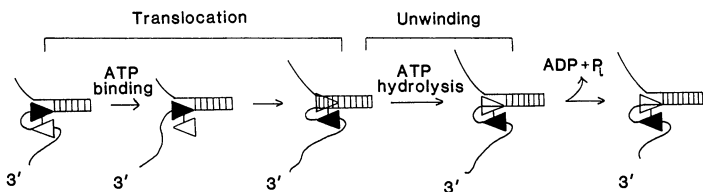
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## Rolling along

During replication double-stranded DNA unwinds to form segments of single-stranded DNA through the action of helicase enzymes. Wong and Lohman (p. 350) explored the mechanistic details of the *Escherichia coli* Rep helicase by comparing the energetics of DNA binding and DNA-induced Rep dimerization for different nucleotide co-factors, ADP (adenosine diphosphate) and AMPP(NH)P, a non-hydrolyzable analog of adenosine triphosphate (ATP). On the basis of these results they propose a rolling model for the unwinding of duplex DNA in which binding of ATP moves the helicase along the DNA; subsequent hydrolysis unwinds several base pairs.



*richia coli* Rep helicase by comparing the energetics of DNA binding and DNA-induced Rep dimerization for different nucleotide co-factors, ADP (adenosine diphosphate) and AMPP(NH)P, a non-hydrolyzable analog of adenosine triphosphate (ATP). On the basis of these results they propose a rolling model for the unwinding of duplex DNA in which binding of ATP moves the helicase along the DNA; subsequent hydrolysis unwinds several base pairs.

## Dating reversals

The record of reversals of the magnetic field preserved in sediments provides a valuable means for dating. However, earlier calibrations, based primarily on K-Ar dating, may be in error. The most recent major reversal, the Brunhes-Matuyama reversal, has also been dated by comparing climatic and paleomagnetic records preserved in sediments to predictions from orbital dynamics. This age is considerably older than the K-Ar age. Baksi *et al.* (p. 356) present a new Ar-Ar age determination of the boundary from lava flows in Hawaii. The new age of  $783 \pm 11$  thousand years ago is in agreement with the astronomically calculated age (see also, *Science*, 8 Nov. 1991, p. 802).

## Charges on contact

Contact or rubbing of dissimilar materials can cause electrical charging, which can be a nuisance (static cling) or a boon (as in photocopying). Despite its importance, this effect has been difficult to understand and study. Horn and Smith (p. 362) modi-

fied the surface forces apparatus, in which surfaces can be brought together and separated with subnanometer accuracy, so that charging and electrostatic forces could be measured simultaneously. The energies required to separate silica and mica after contact charging are nearly as high as their fracture energy. Gas discharges could be observed at distances of about 1 micrometer.

## Water channel structure

Water rapidly enters red blood cells (RBCs) and renal tubes through water channels, whose physiology is well studied but whose structure is unknown. Preston *et al.* (p. 385) microinjected *Xenopus* oocytes with RNA for the CHIP28 protein, an abundant integral membrane protein found in RBCs. The expressed protein conferred increased osmotic water permeability on the oocytes, an activity that could be inhibited with mercuric chloride, a water channel inhibitor. The CHIP28 protein is probably a functional part of the water channel.

## A family affair

Some protein families contain transcription factors that are structurally related. However, each family member may respond to different regulatory signals and function in distinct ways in different cell types. The C/EBP family of proteins belongs to the bZip class of transcription factors. C/EBP $\alpha$  has been shown to function in the adipogenesis of 3T3-L1 cells. The sequence-specific DNA binding protein Myc is involved in the control of cellular proliferation and differentiation. Freytag and Geddes (p. 379) show that expression of Myc in 3T3-L1 adipoblasts inhibits the induction of adipogenesis (formation of fat cells) by C/EBP $\alpha$ . Changes in intracellular calcium levels can result in the activation of specific genes. Wegner *et al.* (p. 370) show that another member of the C/EBP family, C/EBP $\beta$ , is phosphorylated in pituitary cells in response to an increase in intracellular calcium. The calcium-calmodulin-dependent protein kinase II

phosphorylates C/EBP $\beta$  in vitro at Ser<sup>276</sup>. Mutation of this site in C/EBP $\beta$  prohibits calcium-regulated stimulation of a reporter gene containing C/EBP $\beta$  binding sites.

## Chlamydia chromosome

During the biphasic life cycle of *Chlamydia trachomatis*, a sexually transmitted parasite that can cause blindness, the chromosome undergoes organizational changes that appear to be regulated by the expression of Hc1, a protein related to eukaryotic histones. In its extracellular phase, the chromosome is unusually condensed for a prokaryote and does not undergo transcription, but after entering host cells the chromosome is loosely organized and transcription occurs. Barry *et al.* (p. 377) expressed the gene for Hc1 in *Escherichia coli* and observed by microscopy the formation of a condensed nucleoid structure that is similar to that in *Chlamydia*.

## Altered states

One way to increase both the specificity and the repertoire of genes whose expression can be regulated is through interaction among distinct transcription factors. Another means of expanding regulatory possibilities is to generate functionally distinct forms of transcription factors through alternate splicing. Narayanan *et al.* (p. 367) identified a naturally occurring variant of p65, one of the two constituent proteins of the transcription factor NF- $\kappa$ B, that contains a deletion in the transcriptional activation domain (p65 $\Delta$ ). A member of the Rel family of proteins, p65 participates in the transcriptional regulation of viral and cellular genes. Expression of p65 $\Delta$  but not p65 in Rat-1 fibroblasts resulted in focus formation in culture and tumor formation by injection of transformed Rat-1 cells into nude mice. In vitro assays showed that p65 $\Delta$  interfered with DNA binding by p65. Myc is a transcription factor that is involved in the control of cell proliferation, and its binding to DNA is enhanced by the protein Max. Mäkelä *et al.* (p. 373) identified an alternate form of Max ( $\Delta$ Max) that can still bind to DNA in a complex with Myc, but lacks a nuclear localization signal. Expression of Max in rat embryo fibroblasts suppressed transformation, while  $\Delta$ Max enhanced this process.

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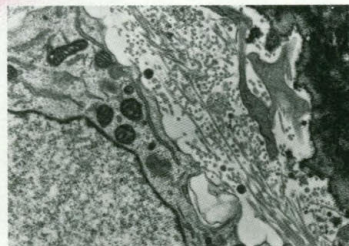
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Photos courtesy of Drs. Warren Ramp and Richard Dillaman.

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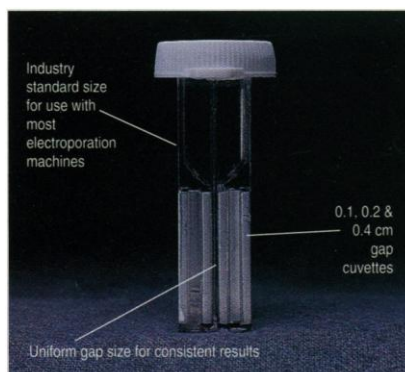
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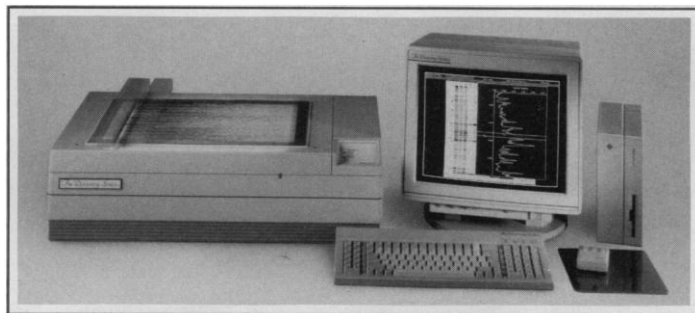
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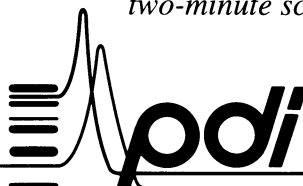
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**For complete guidelines, contact:** Janice Merz, AAAS Behavioral Science Research Prize, American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005, 202/326-6621.



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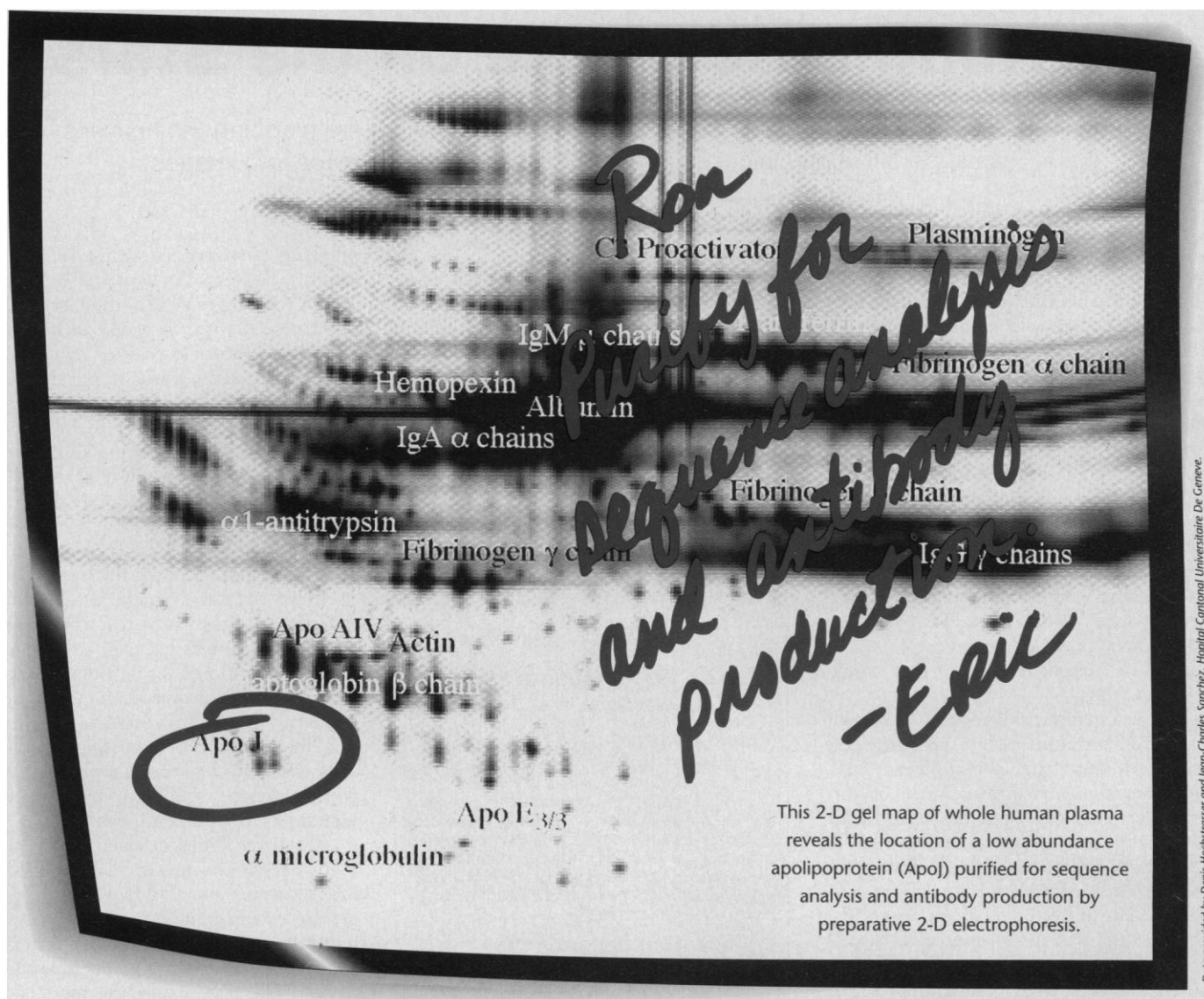
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Application deadline: **June 15, 1992.**

Additional information about applications and travel grants can be obtained from Laura Linzi, International School of Neuroscience, Via Ponte della Fabbrica, 3/A - 35031 Abano Terme (Padova) Italy; Fax 049/810653-810340.

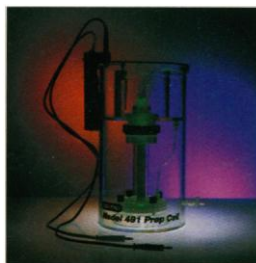
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