



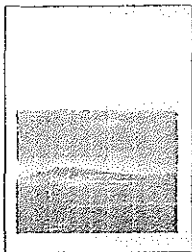
***** CAR-RT SORT ** CR03
25 000009058365C 01/03/92N9103
GEOLOGY GEOPHY LIB
UNIV WISCONSIN
1215 M DAYTON ST
MADISON MI 53706

247 This Week in *Science*

Editorial	249 Teaching and Research
Letters	256 Safety of Bovine Growth Hormone: D. S. KRONFELD; J. C. JUSKOVICH AND C. G. GUYER ■ Interpreting Cancer Tests: J. D. WILSON; G. W. GRIBBLE ■ Kidney Transplantation: Overlooked Pioneer: G. B. ELION
News & Comment	260 The Rush to Publish ■ Lessons from Physics 263 Third Strike for Idaho Reactor 264 CDC Abandons Plans for AIDS Survey Healy Nominated 265 GAO and DOD Get Into a Cat Fight 266 Science Literacy: The Enemy Is Us ■ Science's Top 20 Greatest Hits
Research News	268 New Light on Writing in the Americas 271 Montagnier Pursues the Mycoplasma-AIDS Link 272 Despite Reports of Its Death, the Big Bang Is Safe 274 Global Temperature Hits Record Again 275 <i>Briefings</i> : Radiation Research Shake-Up ■ Private Initiative on Fetal Research ■ U.K. Antes Up for Telescopes ■ George Mason to Set Up Think Tank
Articles	277 Subsistence Economy of El Paraíso, an Early Peruvian Site: J. QUHLER, B. OJEDA E., D. M. PEARSALL, D. H. SANDWEISS, J. G. JONES, E. S. WING 283 Chemistry and Biology of the Immunophilins and Their Immunosuppressive Ligands: S. L. SCHARFBER
Research Article	288 CCAAT-Enhancer Binding Protein: A Component of a Differentiation Switch: R. M. UMIEK, A. D. FRIEDMAN, S. L. MCKNIGHT
Reports	293 An Antimony Sulfide with a Two-Dimensional, Intersecting System of Channels: J. B. PARISE

■ SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005. Second-class postage (publication No. 484160) paid at Washington, DC, and additional mailing offices. Copyright © 1991 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$92. Domestic institutional subscription (51 issues): \$150. Foreign postage extra: Canada \$46, other (surface mail) \$46, air freight \$50. First class, annual, school-year, and student rates on request. Canada GST Number: Pending. Change of address: Allow 6 weeks, giving old and new addresses and 11-digit account number. Postmaster: Send change of address to *Science*, P.O. Box 1723, Riverton, NJ 08077. Single copy sales: \$6.00 per issue prepaid includes surface postage; Guide to Biotechnology Products and Instruments, \$20. Bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of \$1 per copy plus \$0.10 per page is paid directly to CCC, 27 Congress Street, Salem, Massachusetts 01970. The identification code for *Science* is 0036-8075/91 \$11.10. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several special index services.

■ The American Association for the Advancement of Science was founded in 1848 and incorporated in 1874. Its objectives are to further the work of scientists, to facilitate cooperation among them, to foster scientific freedom and responsibility, to improve the effectiveness of science in the promotion of human welfare, to advance education in science, and to increase public understanding and appreciation of the importance and promise of the methods of science in human progress.



COVER Lakes and ponds on the arctic tundra with Irigaknit Mountain in the background, North Slope, Alaska. These aquatic ecosystems are continuously releasing carbon dioxide to the atmosphere. Much of the carbon originates in terrestrial environments, and accounting for this release substantially lowers the estimate of the worldwide arctic sink for atmospheric carbon dioxide. See page 298. [Photograph by George W. Kling]

- 294 Local Structure and Chemical Shifts for Six-Coordinated Silicon in High-Pressure Mantle Phases: J. F. STEBBINS AND M. KANZAKI
- 298 Arctic Lakes and Streams as Gas Conduits to the Atmosphere: Implications for Tundra Carbon Budgets: G. W. KLING, G. W. KIPPHUT, M. C. MILLER
- 301 Putative Skeletal Neural Crest Cells in Early Late Ordovician Vertebrates from Colorado: M. M. SMITH
- 303 Altered Perception of Species-Specific Song by Female Birds After Lesions of a Forebrain Nucleus: E. A. BRENOWITZ
- 305 The Effect of Anti-Neoplastic Drugs on Murine Acquired Immunodeficiency Syndrome: C. SIMARD AND P. JOLICOEUR
- 308 Evidence for Biased Gene Conversion in Concerted Evolution of Ribosomal DNA: D. M. HILLIS, C. MORITZ, C. A. PORTER, R. J. BAKER
- 310 The Effect of the Floor Plate on Pattern and Polarity in the Developing Central Nervous System: S. HIRANO, S. FUSE, G. S. SOHAL
- 313 Regulation of Interleukin-2 Gene Enhancer Activity by the T Cell Accessory Molecule CD28: J. D. FRASER, B. A. IRVING, G. R. CRATREE, A. WEISS

Radical Comments

- 316 Microwave Sounding Units and Global Warming: B. L. GARY AND S. J. KEHM; R. W. SPENCER AND J. R. CHRISTY ■ Lipid Flow in Locomoting Cells: M. S. BREISCHER; K. JACOBSON, J. LEE, M. GUSTAFSSON ■ Bryozoan Morphological and Genetic Correspondence: What Does It Prove?: J. LEVINTON; J. B. C. JACKSON AND A. H. CHEETHAM

Book Reviews

- 324 Authors of Their Own Lives, *reviewed by* A. STCA ■ Australian Ecosystems, M. LOWMAN ■ Thalamic Oscillations and Signaling AND Brainstem Control of Wakefulness and Sleep, C. KOCH ■ Books Received

Products & Materials

- 328 Protein Immunoblotting Incubation Rotator ■ Micromanipulator Table ■ Leiden Microincubator ■ Freezing Stage with Microtome ■ Data Analysis for the Macintosh ■ Monoclonal Antibodies ■ Literature

Board of Directors

Richard C. Atkinson
*Retiring President,
Chairman*

Donald N. Langenberg
President

Leon M. Lederman
President elect

Mary Ellen Avery
Francisco J. Ayala
Eugene H. Cole-Beedes
Robert A. Frosch
Joseph G. Gavin, Jr.
John H. Gibbons
Beatrix A. Hamburg
Florence P. Haselona

William T. Golden
Treasurer

Richard S. Hootson
Executive Officer

Editorial Board

Charles J. Amzlen
Elizabeth E. Bailey
David Bañero
William F. Brinkman
E. Margaret Burbidge
Pierre-Gilles de Gennes
Joseph L. Goldstein
Mary L. Good
Harry B. Gray
F. Clark Howel
Paul A. Marks
Yasutomi Nishizuka
Heen M. Hanesy
Howard A. Schneiderman
Robert M. Solow
Edward C. Stone
James D. Watson

Board of Reviewing Editors

John Abelson
Frederick W. Alt
Don L. Anderson
Stephen J. Benkovic
Gunter K.J. Blobel
Floyd E. Bloom
Henry R. Bourne
James J. Burd
Kathryn Calame
Charles R. Cantor
Ralph J. Ciccerone
John M. Coffin
Robert Dorfman
Bruce F. Erididge
Paul T. Englund
Fredric S. Fay

Harry A. Fozzard
Theodore H. Geballe
Roger I. M. Glass
Stephen P. Goñ
Corey S. Goodman
Stephen J. Gould
Eric F. Johnson
Stephen M. Kosslyn
Konrad B. Krauskopf
Charles S. Lezings III
Richard Losick
John C. McGiff
Anthony H. Means
Mortimer Mishkin
Roger A. Nicoll
William H. Orme-Johnson III
Carl O. Pabo
Yoshayau Pocker

Dennis A. Powers
Erk H. Rugsdahl
Thomas W. Schoneker
Ronald H. Schwartz
Terrence J. Sejnowski
Thomas A. Steitz
Robert T. H. Tjian
Emil R. Unanue
Geerat J. Vermeij
Ben Vogelstein
Harold Weintraub
Zena Werb
George M. Whitesides
Owen N. Witte
William B. Wood
Keith Yamamoto

4. E. P. Lanning, *Peru Before the Incas* (Prentice-Hall, Englewood Cliffs, NJ, 1967).
5. T. C. Patterson, in *Prehistoric Agriculture*, S. Streuver, Ed. (Natural History Press, Garden City, NY, 1971), pp. 181-208.
6. R. Fung P., *Apurimac Archaeol.* 2, 11 (1972).
7. J. H. Steward and L. C. Faron, *Native Peoples of South America* (McGraw Hill, New York, 1959).
8. M. H. Parsons, *Am. Antiq.* 35, 292 (1970).
9. D. J. Wilson, *Am. Anthropol.* 83, 93 (1981).
10. I. S. Raymond, *Am. Antiq.* 46, 806 (1981).
11. J. Quilter and T. Stocker, *Am. Anthropol.* 85, 545 (1983).
12. J. Quilter, *J. Field Archaeol.* 12, 279 (1985).
13. F. A. Engel, *J. Soc. Am.* 55, 43 (1966); *Anal. Cienc. Univ. Agraria* 5, 241 (1967).
14. A. Osborn, in *For Theory Building in Archaeology*, L. Binford, Ed. (Academic Press, New York, 1977), pp. 157-243.
15. S. Quilter suggested this possibility during work at the Paloma Site in 1976.
16. C. E. Smith, in *La Galgada Peru, A Preceramic Culture in Transition*, Terence Grieder et al., Eds. (University of Texas Press, Austin, TX, 1988), pp. 125-151.
17. S. Pozorski and T. Pozorski, *Early Settlement and Subsistence in the Casma Valley, Peru* (University of Iowa Press, Iowa City, 1987).
18. V. Popper, in *Los Gavilanes, Mar, Desierto y Oasis en la Historia del Hombre*, D. Bonavia, Ed. (Editorial Ausonia, Lima, Peru, 1982), pp. 148-156.
19. S. Pozorski and T. Pozorski, *Ann. Carnegie Mus. Nat. Hist.* 49, 337 (1979); J. B. Bird, *Anthropol. Pap. Am. Mus. Nat. Hist.* 62 (1985), part 1.
20. A. Grobman, in *Los Gavilanes, Mar, Desierto y Oasis en la Historia del Hombre*, D. Bonavia, Ed. (Editorial Ausonia, Lima, Peru, 1982), pp. 157-179; R. L. Burger and N. van der Merwe, *Am. Anthropol.* 92, 96 (1990).
21. T. Dillehay, P. Netherly, J. Rosson, *Am. Antiq.* 54, 733 (1989).
22. J. Quilter, "To fish in the afternoon: Beyond subsistence economies in the study of early Andean civilization," paper presented at 51st Annual Meeting of the Society for American Archaeology, New Orleans, LA, 23 April 1986.
23. M. Moseley, *Pre-agricultural Coastal Civilizations in Peru* (Carolina Biology Readers, no. 90, Burlington, NC, 1978).
24. J. Quilter, "Cote and periphery in Pre-ceramic coastal Peru," paper presented at the 88th Annual Meeting of the American Anthropological Society, Washington, DC, 19 November 1989.
25. R. G. Wilkinson, *Poverty and Progress* (Praeger, New York, 1973).
26. Following tax listed in V. Alamo V. and V. Valdivia M., *Bolet. Inst. Mar Peru* (volumen extraordinario, Callito, Peru, 1987).
27. Funding for the El Paraiso research was provided by NSF grant BNS-83-03680, Ripon College Faculty Development Funds, and the Continental Coffee Products Company (a wholly owned subsidiary of Quaker Oats). The excavations were carried out under Credencial 038-83-DCIRBM, issued by the *Instituto Nacional de Cultura* of Peru. We thank A. A. Hunter (Missouri) who identified the squash seeds and A. Price, J. Atchberry, and L. Haubrich who helped in sorting and tallying data. Additional aid in processing the subsistence remains was given by N. Salazar and M. C. Rodriguez de Sandweis in Peru. I. Salazar-Burger, assistant field director, was essential to the project. The *Centro de Investigaciones de Zonas Aridas* was our base of operations and analysis and we thank F. A. Engel and M. Vallejos and many other Peruvian colleagues for support.

Chemistry and Biology of the Immunophilins and Their Immunosuppressive Ligands

STUART L. SCHREIBER

Cyclosporin A, FK506, and rapamycin are inhibitors of specific signal transduction pathways that lead to T lymphocyte activation. These immunosuppressive agents bind with high affinity to cytoplasmic receptors termed immunophilins (immunosuppressant binding proteins). Studies in this area have focused on the structural basis for the molecular recognition of immunosuppressants by immunophilins and the biological consequences of their interactions. Defining the biological roles of this emerging family of receptors and their ligands may illuminate the process of protein trafficking in cells and the mechanisms of signal transmission through the cytoplasm.

RESEARCH DURING THE PAST DECADE HAS CONTRIBUTED significantly to our knowledge of T lymphocyte function. The identification and functional analysis of T cell surface receptors (1) and nuclear transcription factors (2) have made these components of the signal transduction apparatus among the best understood in biology. This understanding is largely due to the use of probe reagents, such as monoclonal antibodies and radiolabeled nucleic acids, that have been developed for the study of surface and nuclear phenomena, respectively. However, the mechanisms for the transduction of signals through the cytoplasm, the "black box" of the signal transduction pathway, remain mysterious.

A family of natural products has emerged as probe reagents for cytoplasmic signaling mechanisms in the T lymphocyte. These small

molecules are immunosuppressants that appear to exert their inhibitory actions distal to early membrane-associated events and proximal to nuclear processes. Studies on a family of immunosuppressant binding proteins, the immunophilins, have attempted to identify the structural requirements for high-affinity interactions between immunophilins and their immunosuppressive ligands and the biological consequences of the formation of immunophilin-ligand complexes. Although there is much to explore in this avenue of research, some general principles associated with the intermediary events of signal processing are emerging.

The Immunosuppressants

Cyclosporin A (CsA), an inhibitor of T cell activation, is currently the favored therapeutic agent for prevention of graft rejection after organ and bone marrow transplantation, and it has been credited with initiating a revolution in clinical transplantation (3-5). The recently discovered compound FK506 inhibits T cell activation by mechanisms that are similar to those of CsA, but FK506 is 10 to 100 times as potent (6). FK506 has performed remarkably well in initial human clinical transplantation trials (7, 8), despite reports of toxic effects in animals (6). Rapamycin inhibits T cell activation at concentrations comparable to those of the structurally related FK506, yet with mechanisms that are strikingly different from those mediated by FK506, and thus CsA (9). Only CsA, FK506, and rapamycin have been used for the identification of members of the immunophilin class. A nonnatural ligand, 506B11 (10), and analogs of CsA (11-13) have also provided insights into the inhibitory mechanisms of immunosuppressants. Many recently discovered immunosuppressive agents (14) with undefined mechanisms, such as

The author is a professor of Chemistry, Harvard University, Cambridge, MA 02138.

discodermolide (15) and deoxypergualin (16), promise to reveal new facets of cytoplasmic signaling mechanisms (17) (Fig. 1).

The Immunophilins

The predominant CsA-binding protein in T lymphocytes is the soluble, cytosolic receptor cyclophilin (18, 19). Cyclophilin is an abundant and ubiquitous protein that is found in both prokaryotic and eukaryotic organisms. The major isoform of human cyclophilin has a mass of 17,737 daltons and an isoelectric point (pI) of 9.3. Two groups have independently reported that cyclophilin is identical to peptidyl-prolyl isomerase (20, 21), an enzyme that catalyzes the interconversion of the *cis*- and *trans*-rotamers of the peptidyl-prolyl amide bond of peptide and protein substrates, and this rotamase activity is potently inhibited by CsA.

Shortly after this discovery, the predominant FK506-binding protein in calf thymus, human spleen, and the T cell line Jurkat, termed FKBP, was isolated and characterized in two laboratories (22, 23). Like cyclophilin, FKBP was shown to have rotamase activity toward a peptide substrate. FK506 inhibits the rotamase activity of FKBP, but not of cyclophilin; likewise, CsA does not inhibit the rotamase activity of FKBP. The cloning (24, 25) and overexpression (24) of human recombinant FKBP and the cloning of an FKBP from *Neurospora crassa* (26) revealed that, despite their common enzymatic properties, FKBP and cyclophilin have dissimilar sequences. Human FKBP has a mass of 11,819 daltons and, like cyclophilin, is a basic (pI = 8.9) (22, 24), cytosolic protein (27). A prokaryotic organism, *Neisseria meningitidis*, was found to have an open reading frame that encodes an FKBP-like protein (24). More recently, FKBP was shown to be the predominant rapamycin-binding protein in yeast, calf thymus, and human T cells (Jurkat) (28). Rapamycin (dissociation constant $K_d = 0.2$ nM) has an even higher affinity for FKBP than does FK506 ($K_d = 0.4$ nM), and is also a potent inhibitor of FKBP's rotamase activity (inhibition

constant $K_i = 0.2$ nM) (29).

Although cyclophilin and FKBP are the only well-characterized immunophilins, other members of this family are known to exist and are currently being investigated. For example, a CsA-binding phosphoprotein of relative molecular mass (M_r) 45,000 has been detected in Jurkat cells (30), and phosphoproteins of M_r 60,000 and 80,000 from this same cell line bind to both FK506 and rapamycin (28). The *nimaA* gene of *Drosophila* (31, 32) and a second cyclophilin-related gene in *Saccharomyces cerevisiae* (33) encode proteins that show high homology to cyclophilin. Several low molecular weight, basic proteins that are retained on CsA, FK506, or rapamycin affinity matrices have also been noted (22, 28). Partial sequence determination of FK506- and rapamycin-binding immunophilins of M_r 30,000 and M_r 13,000 has revealed that these molecules, together with FKBP, are members of a previously unknown family of immunophilins (34). Questions concerning the biological relevance, the rotamase activity, and the affinity to the cognate ligands of these low-abundance immunophilins should soon be answered.

Although the exact cellular concentrations of FKBP and cyclophilin are not known, both are abundant. Saturation binding in the cytosol of Jurkat cells was reported to occur at >5 nM diltiazem-FK506 (27). As FKBP is the predominant cytosolic receptor for drug, this measurement is largely accounted for by FKBP, and thus the cytoplasmic concentration of FKBP may approach 5 nM. The high-affinity FKBP ligands FK506 and rapamycin, however, inhibit T cell proliferation at subnanomolar concentrations (median inhibition concentration $IC_{50} \sim 0.5$ nM) (29, 35). Therefore, inhibition of the rotamase activity of FKBP is very likely an insufficient requirement for mediating the actions of these drugs in T lymphocytes, because only a small fraction of the enzyme would be inhibited at effective drug concentrations. This point has been confirmed by mechanistic studies of FK506 and rapamycin (see below); likewise, investigations of CsA analogs support a similar conclusion regarding the rotamase activity of cyclophilin (12).

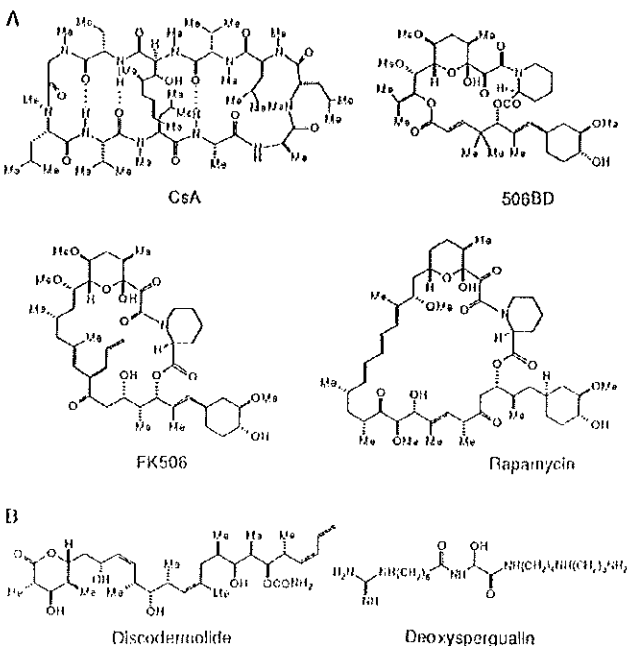


Fig. 1. Probe reagents of intracellular signaling pathways. (A) Recently investigated immunophilin ligands. (B) Immunosuppressive agents with unknown mechanisms of T cell inhibition. (Me, methyl.)

Molecular Recognition by the Immunophilins

The rotamase activity of these immunophilins and the ability of their immunosuppressive ligands to act as rotamase inhibitors provide an opportunity for exploration of the molecular basis for the high-affinity interactions that exist between them. Initial mechanistic studies of cyclophilin led to the suggestion that catalysis of the interconversion of *cis*- and *trans*-rotamers of a peptide substrate is achieved by the formation of a covalent bond to the carbonyl of the peptidyl-prolyl amide with a cysteine-derived thiol (36). Loss of amide resonance would be expected to lower the activation barrier to rotation about the amide C-N bond. Site-directed mutagenesis of human recombinant cyclophilin allowed the systematic replacement of all four cysteine residues in cyclophilin with alanine. Because all four mutants enzymes were fully active in the rotamase and binding assays, cysteine was ruled out as a participating residue in catalysis (37).

Additional mechanistic studies with both cyclophilin (38) and FKBP (39) strongly suggest that these enzymes catalyze rotamer interconversion by noncovalent stabilization of the twisted amide transition state for the noncatalyzed isomerization. The amide functionality exhibits a strong preference for a planar geometry, wherein the nitrogen lone pair is in conjugation with the carbonyl π -cloud. The energy cost of the twisted amide structure (Fig. 2A) is 15 to 20 kcal (40). The structural basis for cyclophilin and FKBP's ability to stabilize this transition-state structure must await further structural analyses of rotamase-peptide (or inhibitor) complexes.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.