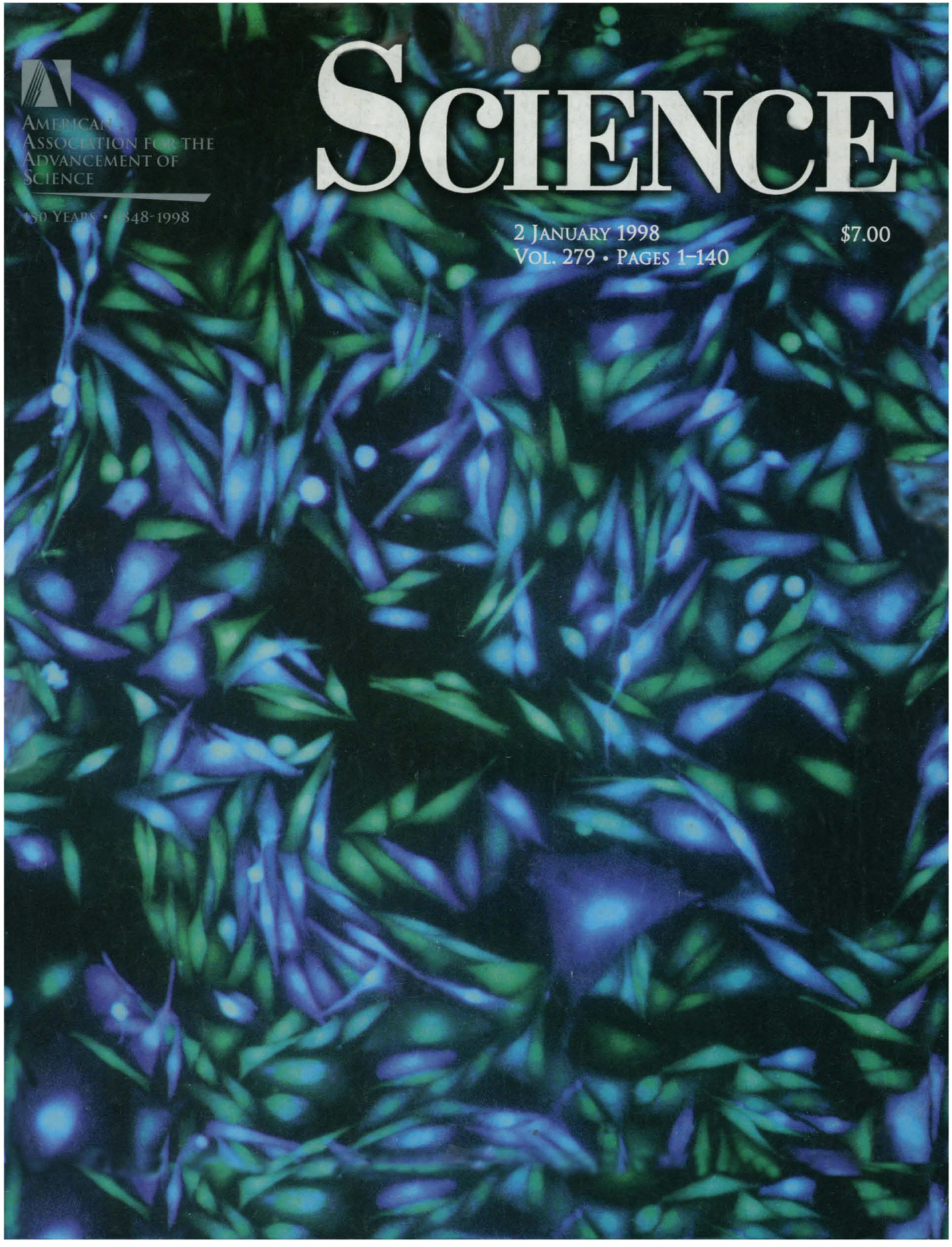
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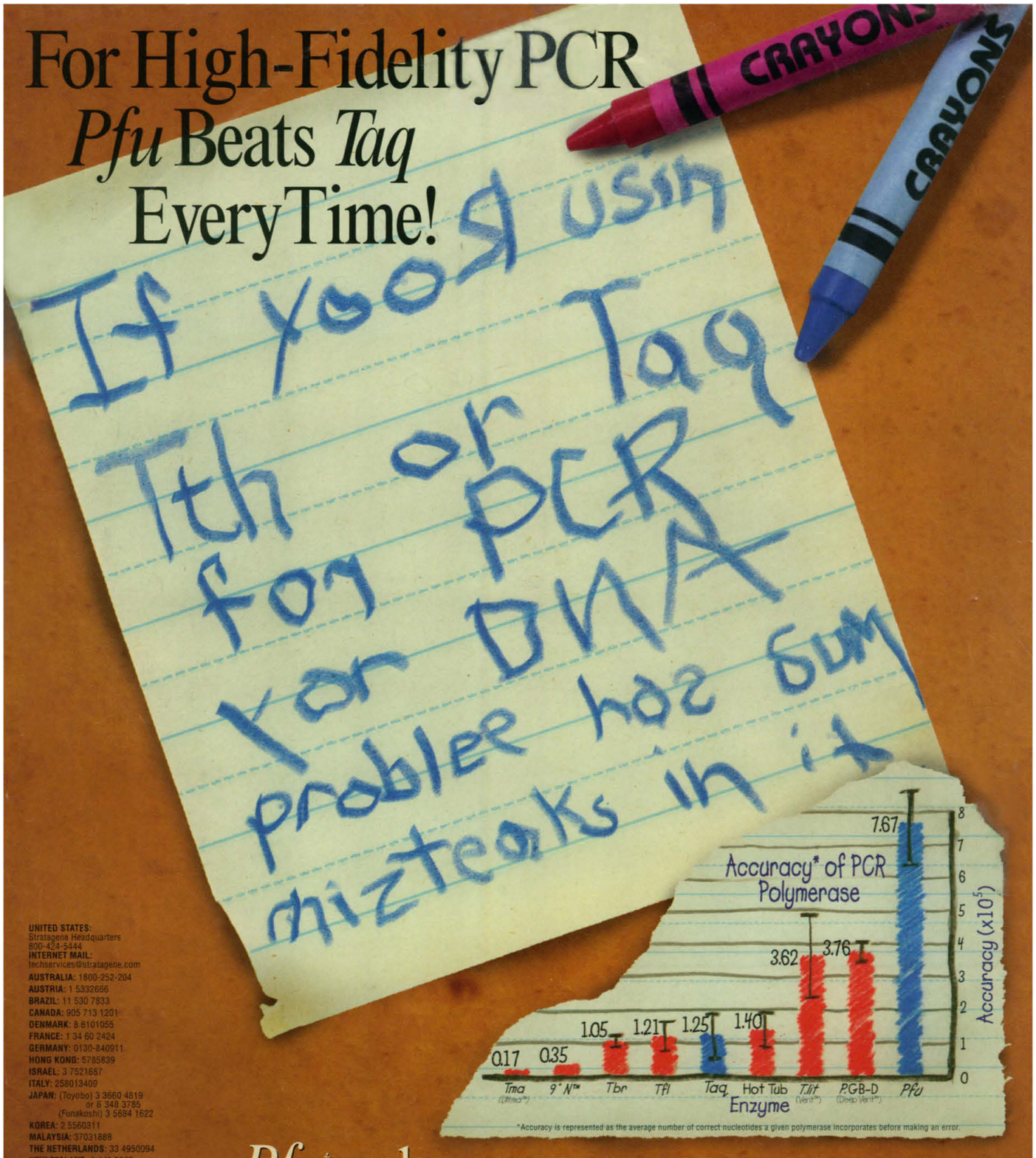

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Painkiller from a frog

98
It fights tuberculosis

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COVER

Gene expression can now be imaged in individual live mammalian cells with higher sensitivity than previously possible. Expression of a β-lactamase reporter gene in Chinese hamster ovary cells is revealed by incubation with a membrane-permeant fluorogenic substrate. Cells expressing β-lactamase fluoresce blue and nonexpressing cells fluoresce green. This technique can be used to clone cells by flow cytometry and to screen drug candidates. See page 84. [Image: H.-C. Tran and G. Zlokarnik]

REPORTS

TECHNICAL COMMENTS

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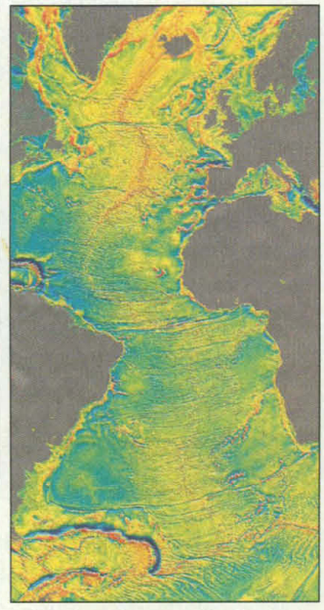

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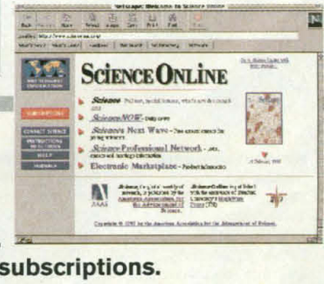
Moving hot spots



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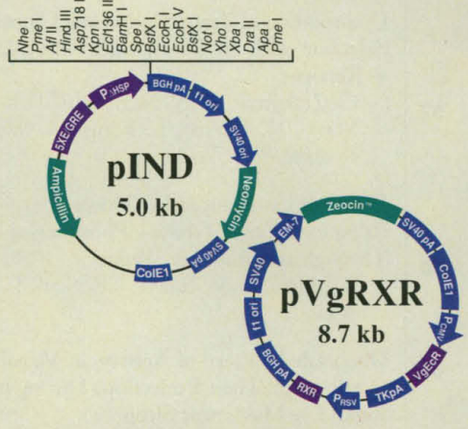
The First Truly Inducible Mammalian Expression System

Invitrogen's new Ecdysone-Inducible Mammalian Expression System is the first system to offer truly low basal expression and high inducibility. Based on a naturally evolved eukaryotic regulatory mechanism that triggers the molting process of insects, ecdysone-responsiveness transfers ideally into mammalian systems (1). This system is entirely unique. It does not rely on prokaryotic operator sites and the artificial nuclear localization of regulatory molecules to control induction as do other regulated systems. This makes the Ecdysone System extremely efficient and easy to use.

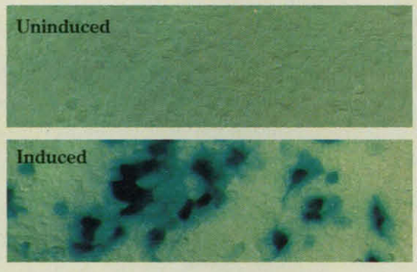
How it Works

For transient transfection and induction, simply clone your gene of interest into pIND, then cotransfect it into mammalian cells with the regulator vector, pVgRXR. Approximately six hours later, treat the transfected cells with the ecdysone analog, muristerone A and begin analysis. Depending on the sensitivity of the assay, you can begin detecting induced expression in as little as two hours. For stable transfection, use dual selection with Zeocin™ and G418.



Tightly Regulated

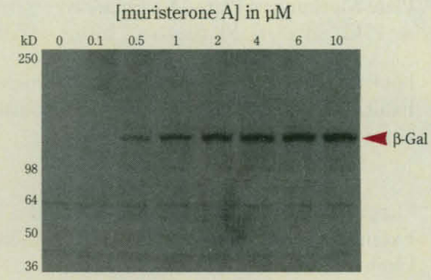
The slides below show a simple colorimetric assay of 293 cells cotransfected with pVgRXR and pIND/*lacZ* before and after muristerone treatment. This example vividly illustrates the Ecdysone System's tight control and capacity for high inducibility.



Uninduced and induced transiently transfected 293 cells stained with X-gal.

Specificity and Control

Induction is specific–exhibiting no pleiotropic effects on mammalian cells–and dose-dependent–allowing for thorough analysis of gene function through a full range of expression.



Dose-dependent β-galactosidase Induction. 293 cells stably transfected with pVgRXR and pIND/*lacZ* treated with increasing amounts of muristerone A. Equal amounts of isolated protein were separated by SDS-PAGE, blotted and detected by antibody and chemiluminescence.

COMPLETE KIT. The Ecdysone-Inducible Mammalian Expression System has everything you need to get started, including vectors, a positive control, sequencing primers, Zeocin™ antibiotic, and muristerone A. Stable cell lines expressing the regulator (pVgRXR) are also available. Call Invitrogen or visit our web site to learn more about the Ecdysone-Inducible Mammalian Expression System.

1. No, D. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**: 3346-3351.

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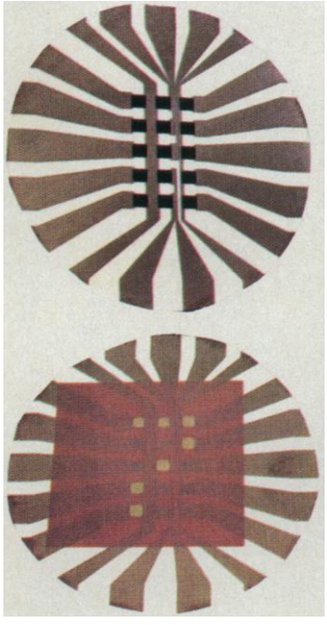
edited by PHIL SZUROMI

Molecular memory

Molecules or molecular assemblies may be used as single bits of information if they can be switched between different states in a highly controlled manner and the system has a different



optical or magnetic response in the two states. Kahn and Martinez (p. 44) review how compounds can be designed that exhibit a cooperative transition between a low-spin and high-spin electronic state. The compounds are polymerized chains of organometallic clusters; cooperative interactions between units in the chain enhance the response compared to separate cluster compounds. The transition temperatures can be tuned by changing compositions. These spin-transition polymers may have applications as temperature sensors, elements of displays and in information storage and retrieval.

■

Imaging with polymers

Photorefractive materials change their refractive index upon irradiation with light of an appropriate wavelength and can be used, for example, to record information optically in three dimensions. Infrared photorefractive polymers are particularly desirable for medical imaging applications. Kippelen *et al.* (p. 54; see the news story by Service, p. 33) followed recently proposed design guidelines and synthesized and characterized a molecule that is photorefractive in the near infrared. The overall performance of the material allows imaging through scattering media at a wavelength compatible with biological tissues.

■

Polar films

Ultrathin molecular layers with stable polar order have potential application in electric and electro-optical devices, but obtaining large persistent polarization (orientation of dipoles) can be difficult to achieve. Jaworek *et al.* (p. 57) measured the electromechanical properties of a polymer film formed by helical polyglutamates grafted to a flat aluminum surface. Ultrathin polypeptide layers with large persistent polarization were formed, without the need for subsequent poling. The films can be grown directly on a variety of electrodes and flexible substrates.

■

A stable, magnetic core

Earth's magnetic field is assumed to be controlled by the motions of the fluid, iron-rich outer core, but numerical simulations also indicate that the solid, iron-rich inner core is conductively coupled to the outer core and helps to maintain a stable field. Gilder and Glen (p. 72) determined the magnetic properties of hexagonal closed-packed iron for pressures up to 16.9 gigapascals and temperatures up to 260° Celsius. If these properties can be extrapolated to the higher pressures and temperatures of the inner core, then their work suggests that the inner core iron may be paramagnetic. This property would keep the magnetic field from reversing because of short-term fluctuations in the outer core, thus providing a needed stabilizing mechanism.

■

Extended consequences

The Basin and Range province of the western United States is a key area for studying extension of Earth's crust. During the last 50 million years, the crust here extended laterally in some cases by more than 100 percent. Extension in many places was along enigmatic low-angle normal faults and was associated with volcanic activity. Gans and Bohrson (p. 66) show paradoxically that volcanic activity waned abruptly in several areas when the most rapid extension began, rather than being facilitated by the extension. Morrison and Anderson (p. 63) show in one area, the Whipple Mountains, that the faulting may have allowed an influx of cool surface waters that refrigerated the top of the lower crust below the fault.

■

Mysterious sources and sinks

The hydrogen radicals OH and HO_2 are crucial oxidants in atmospheric chemistry throughout the troposphere. However, their relatively low concentrations make direct atmospheric measurements quite difficult. Wennberg *et al.* (p. 49) have performed aircraft measurements of a range of compounds, including OH and HO_2, in the upper troposphere. Detailed modeling studies show that all of the known sources and sinks cannot account for the measurements and that additional sources must be present. The results also affect the ozone chemistry of the upper troposphere: The production rate of ozone is faster than previously assumed in this region of the troposphere, and input of NO (for example, by air travel) will have a greater effect on ozone concentrations than expected.

■

Mantle mountain building

The coupling of tectonic plate movements to convective circulation in the mantle is difficult to correlate directly in many regions. Silver *et al.* (p. 60), through a detailed study of surface deformation and a model of plate velocities triggered by mantle flow, have correlated the movements of the South American and African plates with an anomalous mantle upwelling. The changes in plate velocities triggered by this coupled motion helps to explain the development of the Andean and the Alpine-Himalayan mountain belt systems around 30 million years ago.

■

Migrating planets

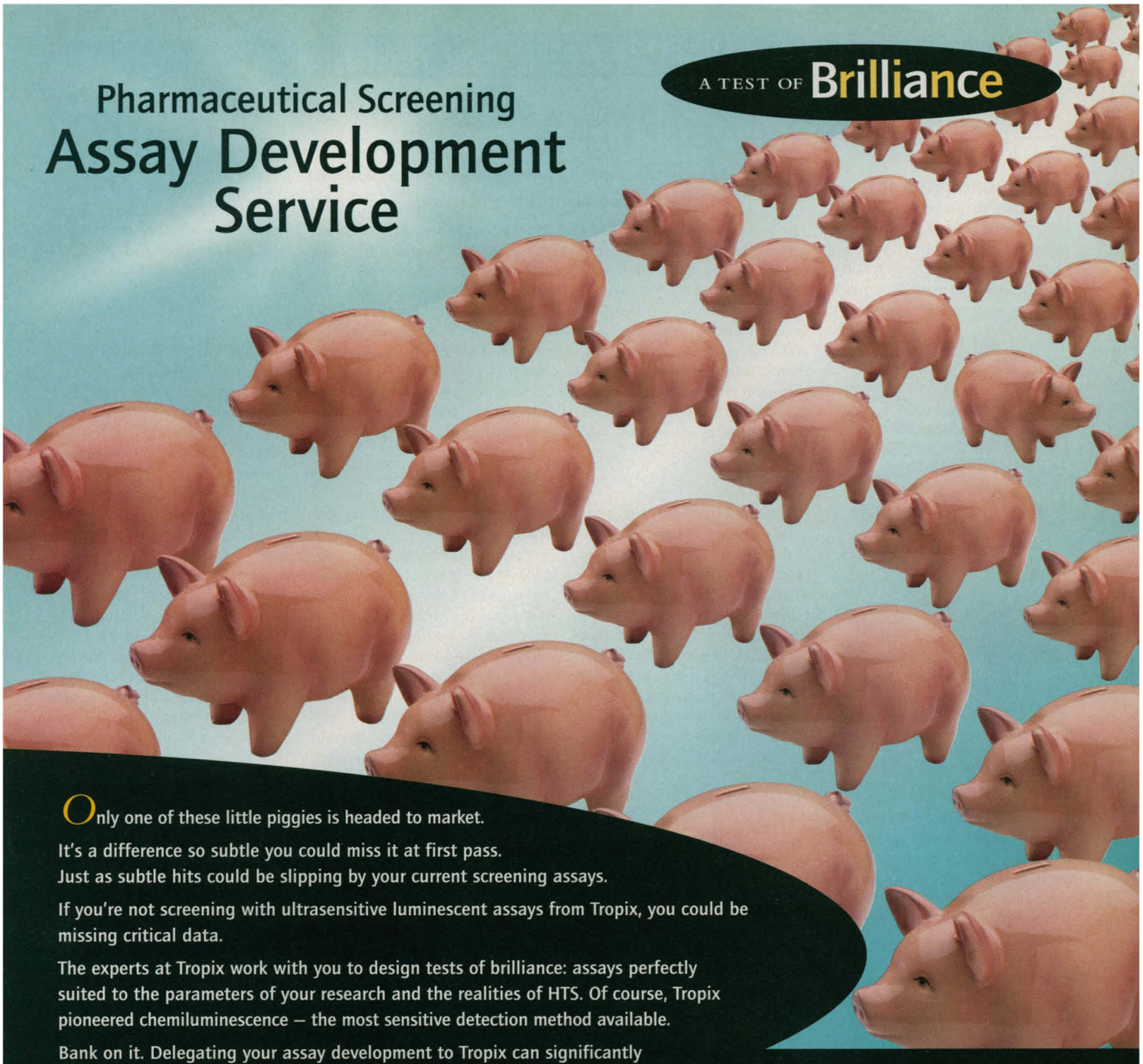
The recent discoveries of Jupiter-mass planets orbiting close to their central stars in other solar systems creates a problem of how these gas giants got so close to the central star, given that there is no known formation method that would allow such extrasolar planets to initially form there. Murray *et al.* (p. 69) suggest that a giant planet forms in the outer part of its system and then migrates toward the star. The planet is pushed toward the star through instabilities created by planetesimals that linger in the disk after most of the gas has been consumed in planet formation.

■

Salted atmosphere

Halogen atoms (such as chlorine and bromine) could be an important reactant in the Earth's atmosphere and greatly affect tro-

(continued on page 9)



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(continued from page 7)

pospheric ozone levels, but the rate and processes of formation of chlorine gas from sea salt, the most likely global source of halogen atoms, have been uncertain. Oum *et al.* (p. 74) show that Cl_2 can be generated by photolysis of ozone in the presence of sea salt. These results suggest that there is a likely important marine source of chlorine.

■

Selenium in a viral protein

The poxvirus molluscum contagiosum causes both skin cancers in children and opportunistic infections in individuals with AIDS. The virus itself is difficult to study because it does not replicate in tissue culture and only minimally in animal models. Shisler *et al.* (p. 102; see the commentary by McFadden, p. 40) found an open reading frame in the virus that encodes a selenocysteine insertion sequence that allows translation of a selenium-containing protein, glutathione peroxidase. Neither selenoproteins nor this type of translational control had been seen previously in a virus. When the selenoprotein was transfected into human keratinocytes, it was able to protect them from the cytotoxic effects of ultraviolet irradiation and hydrogen peroxide. The viral glutathione peroxidase may protect infected cells from oxidative stresses induced by the host immune system during inflammatory responses.

■

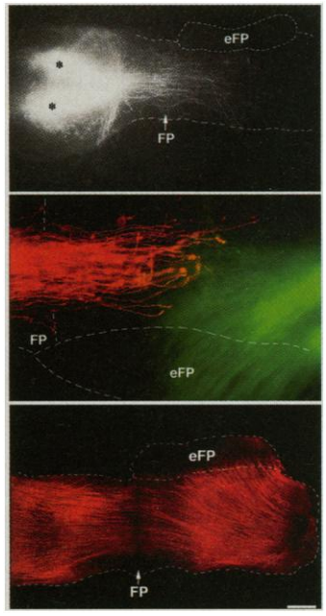
Potent, nonaddictive analgesic

Analgesics such as morphine can suppress severe pain, but they also cause serious side effects, including dependence and withdrawal symptoms, that often limit the number of situations in which they are used clinically. Bannon *et al.* (p. 77; see the news story by Strauss, p. 32) describe a compound that acts as a potent analgesic—equivalent to morphine—in several independent animal models of pain, but appears to lack the tendency to cause dependency or withdrawal.

■

Ignores the guide

If chemoattractive signals tell developing axons to extend toward the embryonic midline, how do those same axons ever leave



the midline and depart for the other side? Shirasaki *et al.* (p. 105) show that axons destined to cross the vertebrate spinal floor plate lose sensitivity to the chemoattractant once they reach the cells of the floor plate. Axonal navigation through a complex route may require a series of regulated changes in sensitivity to various signals.

■

In our wildest dreams

Many people have experienced dreaming during the rapid eye movement (REM) phases of sleep. Braun *et al.* (p. 91) have conducted a functional imaging study to examine patterns of activity in the visual processing pathways during REM sleep. They find that while the earliest stages (striate cortex) and highest processing centers (prefrontal cortex) are quiescent, the intermediate extrastriate areas and limbic system are active, consistent with the common emotionality of dreams.

■

In the beginning

Analyses in yeast have offered great insight into the specific molecular interactions that lead to chromosomal DNA replication. Bielinsky and Gerbi (p. 95) have now precisely mapped the start sites from which DNA replication begins at the yeast *ARS1* origin. Through the use of replication initiation point mapping, they identify the location at which continuous and discontinuous DNA replication meet. The location is adjacent to the binding site for the origin recognition complex.

■

Tuberculosis drug target

Isoniazid (isonicotinic acid hydrazide) is the most commonly used drug against *Mycobacterium tuberculosis*. An activated form of this drug inhibits its target, InhA, an enoyl-acyl carrier protein reductase that uses nicotinamide adenine dinucleotide (NADH) as a cofactor. Rozwarski *et al.* (p. 98) performed mass spectrometric and crystallographic studies which show that the activated form of the drug covalently attaches to the nicotinamide ring of the NADH bound in the active site of the enzyme. These studies may allow the development of drugs that bypass activation by a catalase-peroxidase (KatG) that has been implicated in drug resistance.

■

Color test for transcription

Real-time visual detection of transcription in single mammalians transfected with a bacterial TEM-1 β-lactamase gene has been performed by Zlokarnik *et al.* (p. 84; see the cover), who designed a cell-permeable substrate that, when cleaved by the β-lactamase, changes its fluorescence from blue to green. The high sensitivity of the system, which requires expression of less than 100 molecules of β-lactamase to produce a shift in color detectable by eye, should allow the study of expression of endogenous genes with unmodified promoters.

Technical Comment Summaries

Polar Wander and the Cambrian

J. F. Kirschvink *et al.* proposed (Reports, 25 July, p. 541) that key continental movements during the Cambrian "were driven by an interchange event in Earth's moment of inertia tensor," that is, by true polar wander (TPW).

T. H. Torsvik *et al.* state that, although the TPW idea is "intriguing," an analysis of a "more complete data set" (derived from geomagnetic samples from several studies) is consistent with "conventional plate tectonic systematics."

In response, Kirschvink and co-workers (Evans *et al.*) state that their conclusion is based on "a more reliable subset of the data" and note that the TPW hypothesis neatly explains many features of the Early Cambrian geologic record.

For the full text of these technical comments see: www.sciencemag.org/content/279/5347/9a

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EDITORIAL & NEWS CONTACTS

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