Retraction

I WISH TO RETRACT THE RESEARCH ARTICLE "Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing" (*1*). My laboratory is unable to reproduce the observations reported in Figs. 1 and 2 because we do not possess the plasmid pshura4SE-280 plasmid or fission yeast strains that contain it. In addition, attempts to reproduce these observations with other ura4-hairpin constructs have failed. I therefore retract the conclusion that expression of a hairpin RNA homologous to ura4 RNA results in the production of siRNA that bring about methylation of histone H3 on lysine 9 and recruitment of Swi6 to the ura4$^+$ gene in *Schizosaccharomyces pombe*. In addition, the data (Figs. 3, 4, and 5) indicating that long terminal repeats (LTRs) are enriched in H3K9me2 and Swi6 chromatin and that the expression of genes lying close to LTRs in cells lacking Ago1, Dcr1, Rdp1, Clr4 or Swi6 is increased are not reproducible by us or others. I therefore retract the conclusion that LTRs in *S. pombe* are packaged in RNAi-dependent heterochromatin and that these LTRs are involved in the regulation of nearby genes. I deeply regret any inconvenience that publication of these data has caused for others. The first author of the original paper, Vera Schramke, declined to be an author of this retraction.

ROBIN ALLSHIRE

Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology, King's Buildings, University of Edinburgh, Edinburgh EH9 3JR, UK.

Reference

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Benefits and Risks in Malaria Control

THE REPORTS BY S. BLANFORD *ET AL.* ("Fungal pathogen reduces potential for malaria transmission," 10 June, p. 1638) and E.-J. Scholte *et al.* ("An entomopathogenic fungus for control of adult African malaria mosquitoes," 10 June, p. 1641) identify the fungus *Metarhizium anisopliae* as a potentially useful pesticide to combat malaria. For over a century, *M. anisopliae* has been used for agricultural pest control. To date, it has not been demonstrated to be either infectious or toxic to mammalian species. However, our studies (*1*–*3*) have demonstrated that mice exposed via the respiratory tract to extracts of *M. anisopliae* developed responses similar to those of human allergic asthmatics. Additionally, there is anecdotal information as well as limited clinical data suggesting that some individuals exposed occupationally to *M. anisopliae* have become sensitized (*4*). The "active ingredient" for commercial application of *M. anisopliae* is the conidium (asexual spore), a viable reproductive unit, which is likely to spread, particularly because *M. anisopliae* infects a wide range of insects. In indoor environments, genetically predisposed individuals exposed to spores as well as to mycelium (hyphae) and the inducible proteases and chitinases may develop an allergic reaction potentially leading to asthma. The lives saved by mosquito control are an important goal. However, it is important to be aware that there are risks associated with the possibility of repeated spraying in indoor environments and the fact that allergenicity does not necessarily depend on viability of spores.

MARSHA D. W. WARD* AND MARY JANE K. SELGRADE*

U.S. Environmental Protection Agency, Immunotoxicology Branch, 109 TW Alexander Drive, Research Triangle Park, NC 27711, USA.

*This letter does not reflect EPA policy.

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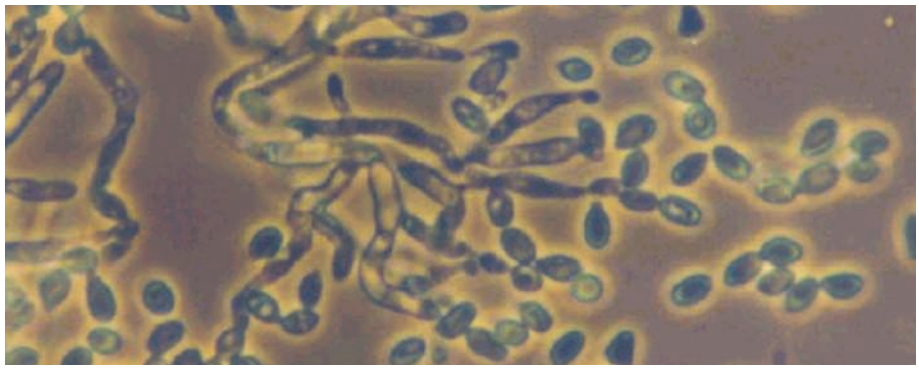
S. BLANFORD *ET AL.* ("FUNGAL PATHOGEN reduces potential for malaria transmission," Reports, 10 June, p. 1638) and E.-J. Scholte *et al.* ("An entomopathogenic fungus for control of adult African malaria mosquitoes," Reports, 10 June, p. 1641) describe the use of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* as agents for mosquito vector control to combat malaria. Scholte *et al.* hung *M. anisopliae*–impregnated sheets within houses in Tanzania. A similar vehicle of delivery is proposed for *B. bassiana*. Although we welcome new weapons in the war against malaria, potential side effects should be addressed before their deployment. Two concerns about the use of these entomopathogenic fungi for mosquito control have already been raised: the indiscriminate killing of insects within treated dwellings, and evolutionary effects on *Plasmodium* development (*1*). We have further concerns about this proposed mosquito control method. Some insect groups, such as locusts, ants, and termites, can become resistant to *M. anisopliae* infection (*2*–*4*). The levels of mosquito mortality achieved by Blanford *et al.* (~90%) could allow the selection of fungus-resistant flies. Also, it takes several days before infected mosquitoes die, during which time they are likely to disperse with the fungi. Previously, these entomopathogenic fungi have been shown to kill a wide variety of invertebrates, but also fish and reptiles (*5*–*7*). In addition, *M. anisopliae* is toxic to two indigenous African Sahel invertebrates, the fairy shrimp *Streptocephalus sudanicus* and the backswimmer *Anisops sardeus* (*8*). Worryingly, *B. bassiana* can infect immunosuppressed people (*9*, *10*), a particular concern in many of the malaria-endemic regions of sub-Saharan Africa with high HIV/AIDS prevalences. Negative ecological impacts need to be fully evaluated and weighed against achievable human health benefits.



Metarhizium anisopliae

OLIVER C. HUTCHINSON AND ANDREW A. CUNNINGHAM

Institute of Zoology, Regent's Park, London NW1 4RY, UK.

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Response

ANY NOVEL ANTIMALARIA TECHNOLOGIES must be subject to rigorous public health cost-benefit evaluations. Given the magnitude of the malaria problem, and our current understanding of these insect pathogens, we are cautiously optimistic that the use of fungal biopesticides for malaria control will not be curtailed by their theoretical potential to impact on human or environmental health in malaria-endemic regions.

Any small protein-based particles can act as allergens. Such aeroallergens are ubiquitous (*1*). Similarly, common infectious fungal pathogens such as *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. (*2*) are abundant and widespread. It seems unlikely that antimalaria fungal biopesticides would add significantly to this burden. The delivery systems we are investigating use spores formulated in oil. These oil formulations cause the spores to adhere to the substrate on which they are applied and so should not contribute substantially to spore load in the air. We expect the majority of infected mosquitoes to die in the external environment. Studies on the effects of *M. anisopliae* on grasshoppers in Africa indicate that >95% of infected individuals can be preyed upon before they ever have a chance to produce new spores (*3*). Some mosquitoes may, of course, die in houses, but spores only appear on insect cadavers when ambient relative humidity is very high, and by the time any spores could appear, scavengers such as ants will likely have removed any infected corpses that have evaded routine house cleaning.

Regarding broader environmental impact, possible nontarget species within houses, such as flies and other mosquito species, are frequently nuisance pests and sometimes also associated with health risks. Moreover, contrary to the suggestion of both sets of Letter writers, fungal host range is isolate-specific, not species-specific (*4*); thus, we as yet know little about host specificity of the individual fungal isolates we are investigating. Furthermore, infection also depends on ecological context. The study on the impacts of *M. anisopliae* on aquatic invertebrates cited by Hutchinson and Cunningham, for example, used a maximum challenge test exposing organisms to high doses of spore powder (of a completely different isolate) mixed in water with detergent (*5*). Such an exposure route is of questionable relevance given our intended use strategy.

No interventions are risk-free, and it's important to evaluate what these risks are. Indeed, regulatory frameworks rightly demand it. However, risks should not be viewed in isolation or evaluated without proper appreciation of both the ecological and socioeconomic contexts.

MATT B. THOMAS,[1*] SIMON BLANFORD,[2] NINA E. JENKINS,[3] GERRY F. KILLEEN,[4,5] BART G. J. KNOLS,[6,7] ANDREW F. READ,[2] ERNST-JAN SCHOLTE,[7] WILLEM TAKKEN[7]

[1]Imperial College London, Wye Campus, Wye, Kent TN25 5AH, UK. [2]Institutes of Evolution, Immunology and Infection Research, University of Edinburgh, Edinburgh EH9 3JT, UK. [3]CABI Bioscience at Department of Agricultural Sciences, Imperial College London, Wye Campus, Wye, Kent, TN25 5AH, UK. [4]Ifakara Health Research and Development Centre, Post Office Box 53, Ifakara, Tanzania. [5]Department of Public Health and Epidemiology, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland. [6]International Atomic Energy Agency (IAEA), A-2444 Seibersdorf, Austria. [7]Laboratory of Entomology, Wageningen University and Research Centre, Post Office Box 8031, 6700 EH, Wageningen, The Netherlands.

*To whom correspondence should be addressed. E-mail: m.thomas@imperial.ac.uk

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Estrogen Receptors and Cell Signaling

THE REPORT BY C. M. REVANKAR *ET AL.* ("A transmembrane intracellular estrogen receptor mediates rapid cell signaling," 11 Mar., p. 1625) presents findings that the G protein–coupled receptor GPR30 localizes exclusively to endoplasmic reticulum and binds an estradiol-Alexa dye conjugate. As such, they suggest that it signals so-called estrogen-induced "rapid effects" in estrogen receptor (ER)–negative cells. In the accompanying Perspective ("A new mediator for an old hormone?", 11 Mar., p. 1572), S. C. Hewitt *et al.* note that there are earlier reports of estrogen-induced GPR30 signaling, but in these studies, GPR30 was localized in the plasma membrane, not the endoplasmic reticulum; other work suggests GPR30-mediated estrogen effects

but at μM levels of hormone (*1*), not the nM levels seen by Revankar *et al*. Further, the GPR30 gene may not be expressed in most ER-negative breast cancers (*2*). Also, Ahola *et al.* (*3*) find that estrogen induces growth in cells treated with GPR30 antisense oligonucleotides, indicating that GPR30 is not required for this action. The PI3K assay used by Revankar *et al.* addresses PIP3 accumulation in nuclei, but it is not clear if this means PI3K is activated at the plasma membrane by GRP30. There is no report of high-affinity, saturable estrogen binding to the endoplasmic reticulum. Another difficulty is the plethora of ER forms; some cells once considered ER-negative express ERβ or variant ERs. Also, the biological significance of estrogen signaling in ER-negative breast cancers is unknown. Clinical findings on treatment of breast cancer patients show significant benefit of hormonal therapy in those with ER-positive tumors, but little in those with ER-negative tumors (*4*). Evidence suggests that ER associates with the plasma membrane and is required for rapid responses to estradiol (*5*, *6*). No proof has yet been provided that GPR30 is relevant to estrogen action in ER-positive breast cancer. A role for GPR30 in cellular actions of estrogen, as emphasized by Revankar *et al.* in ER-negative breast cancer cells, remains to be demonstrated.

RICHARD J. PIETRAS,[1]* ELLIS R. LEVIN,[2] CLARA M. SZEGO[3]

[1]Department of Medicine-Hematology/Oncology and Jonsson Comprehensive Cancer Center, University of California, Los Angeles School of Medicine, Los Angeles, CA 90095–1678, USA. [2]Departments of Medicine, Biochemistry and Pharmacology, University of California, Irvine School of Medicine, Irvine, CA 92717, USA. [3]Department of Molecular, Cell & Developmental Biology and Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90095–1606, USA.

*To whom correspondence should be addressed. E-mail: rpietras@ucla.edu

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Response

PIETRAS *ET AL.* SUGGEST THAT IN EARLIER reports of estrogen-induced signaling, GPR30 was shown to localize to the plasma membrane. However, the original description of GPR30 function (*1*) did not examine the receptor's localization; rather, it was assumed that it would be expressed in the plasma membrane. Furthermore, an

additional study (*2*) provided no markers by which to establish the subcellular localization of the diffuse cellular GPR30 staining. The use of membrane preparations, at times designated as plasma membranes, for binding and functional assays must also be viewed in light of the fact that the preparations were either crude (*3*) or at best enriched with marker distributions not presented to demonstrate purity (*2*). Our conclusion that GPR30 is expressed in the endoplasmic reticulum is supported by multiple independent approaches. Pietras and colleagues infer that other work (*4*) suggests that GPR30-mediated estrogen effects occur only at μM and not nM concentrations of estrogen. Robust GPR30-dependent cellular responses at 1 nM estradiol have been demonstrated [(*1*, *5*–*7*); our Report]. In addition, the selectivity of 17β-estradiol over 17α-estradiol has been shown to be in excess of 10^3 to 10^4 [(*2*); our Report], revealing high selectivity for the physiological isomer of estradiol. Pietras *et al*. question whether the nuclear PIP3 accumulation is due to PI3K activation—our data demonstrate the de novo synthesis of PIP3 by PI3K. With respect to estradiol binding capacity, we demonstrated that binding of estradiol-Alexa633 to GPR30 is essentially equimolar with binding to ERα and ERβ (Fig. 2E). Finally, competition binding studies with 17β-estradiol demonstrated a K_i of ~6 nM, within twofold the value recently reported by others using tritiated estradiol (*2*).

As Pietras *et al*. point out, some cells previously considered ER-negative may express ERβ or other ER variants. For these reasons, we expressed GPR30 in cells that are otherwise unresponsive to estrogen stimulation and in addition, we depleted GPR30 in cells expressing endogenous GPR30 to confirm that the only estrogen-responsive receptor in the cells is GPR30. We agree that recent evidence suggests that the classical ER can associate with the plasma membrane and initiate rapid signaling, confirming the multiple mechanisms through which the classical estrogen receptor is likely to mediate the effects of this hormone. Such results, however, do not exclude a possible role for GPR30 in estrogen biology.

ERIC R. PROSSNITZ,[1,2]* CHETANA M. REVANKAR,[1,2] JEFFREY B. ARTERBURN,[4] LARRY A. SKLAR[2,3]

1Department of Cell Biology and Physiology, 2Cancer Research and Treatment Center, 3Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, USA. 4Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA.

*To whom correspondence should be addressed. E-mail: eprossnitz@salud.unm.edu

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What Should We Call Pluto?

THE DISCOVERY OF 2003 UB313, AN OBJECT that is larger than and farther away from the sun than Pluto, has reopened the question of what is a planet ("Newfound 'tenth planet' puts Pluto behind the eight ball," R. A. Kerr, News of the Week, 5 Aug., p. 859). The International Astronomical Union (IAU) has suggested the idea of calling both Pluto and the new object "Trans-Neptunian planets" (TNPs), including planet in the name but with a qualifier. This proposal is worth a try, but it raises some problems. Pluto, UB313, and other similar bodies must be named as asteroids exclusively, not only because they are different from the other eight planets, but because of their similarity in origin, size, composition, and orbital parameters to hundreds of thousands of small rocky objects orbiting the sun at more than 30 AU, in a region called the Edgeworth-Kuiper Belt. If the term "planet" is still included in the name, then we could have hundreds of this new kind of planet in the solar system in the future. If TNPs are a class roughly defined as "large objects that orbit the sun beyond Neptune," what will be the name of similar small rocky objects that will eventually be discovered orbiting in the outer regions of stellar systems other than ours?

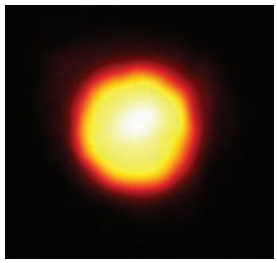

Image of Pluto taken by the European Space Agency's Faint Object Camera on 21 February 1994, when Pluto was 4.4 billion km from Earth.

CREDIT: R. ALBRECHT, ESA/ESO SAPCE TELESCOPE EUROPEAN COORDINATING FACILITY; NASA

In planetary science, the tools for examining our cosmic neighborhood continue to be refined and extended, and so we are beginning to appreciate that the richness of our stellar system is much greater than it appeared to earlier explorers and name-givers, demanding revisions of

which Pluto is an excellent example. As Confucius said, "The beginning of wisdom is to call things by their right name."

ALBERTO G. FAIRÉN
Centro de Biología Molecular, Universidad Autónoma de Madrid, Facultad de Biología, Laboratorio C-101, Cantoblanco-Madrid 28049, Spain.

CORRECTIONS AND CLARIFICATIONS

Table of Contents: (2 Sept., p. 1447). The Brevia "Major biocontrol of plant tumors targets tRNA synthetase" by J. S. Reader *et al.* should have been listed with the overline of microbiology, and the one-sentence summary should have read, "A biocontrol agent for crown gall tumors acts by inactivating the transfer RNA synthetase for leucine, an approach that might be useful in targeting other plant diseases."

Reports: "Cladosporium Avr2 inhibits tomato Rcr3 protease required for *Cf-2*–dependent disease resistance" by H. C. E. Rooney *et al.* (17 June, p. 1783). Reference (*28*) was cited three times in the Fig. 1 legend, once in the Fig. 2 legend, and once in the Fig. 3 legend. All of these citations should have referred to reference (*29*), the Materials and Methods section of the Supporting Online Material. In addition, on p. 1786, *Pseudomonas syringae* p. *maculicola* should be *Pseudomonas syringae* pv. *maculicola*. The correct sentence should read, "In the case of RPM1- and RPS2-mediated resistance in *Arabidopsis*, the action of the Avr proteins AvrB, AvrRpm1, and AvrRpt2 on the guardee RIN4 is thought to trigger the activation of the RPM1 (resistance to *Pseudomonas syringae* pv. *maculic0la* expressing *AvrRpm1*) or RPS2 (resistance to *Pseudomonas syringae* pv. *tomato* expressing *AvrRpt2*) proteins (*24*–*27*)."

Reports: "The effects of artificial selection on the maize genome" by S. I. Wright *et al.* (27 May, p. 1310). In the first full paragraph of the third column on page 1312, the value of the likelihood ratio (LR) is incorrect. The sentence should read, "The LR provides statistically significant support for the presence of two gene classes (LR = 6.35, df = 2, $P < 0.05$)." This correction does not modify the results or the conclusions in any way. In addition, in the first column of page 1313, the LR statistic should read "(LR = 4.6, df = 2, $P = 0.10$, $\hat{k}_1 = 2.45$, $\hat{k}_2 = 0.001$)." With this correction, statistical support for the selection model with the reduced data set is borderline significant. However, the author's best estimate of f based on the reduced data remains 3.6%, and this correction does not substantially modify the results or the interpretation, since statistical support for selection was obtained from the full data set.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.