Time for a change

Prokaryote: gene-sequence comparisons show the tree of life consists of bacteria, eukarya and archaea. The use of the term 'prokaryote' fails to recognize that an idea about life's origins has been proved wrong.

Norman R. Pace

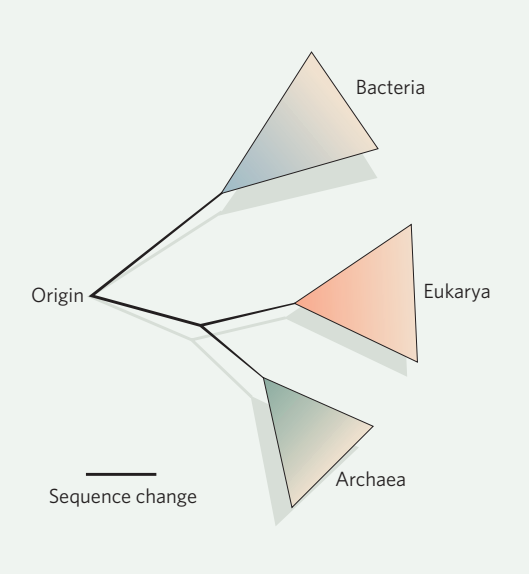
The explosive accumulation of gene sequences over the past few decades has brought a new perspective on life and its history. Some of the results indicate that we need to reassess our understanding of the course of evolution at the most fundamental level.

The current textbook paradigm for biological diversity and evolution is based on what I will call the prokaryote/eukaryote model. This posits that there are two kinds of cells: prokaryotic, those without nuclei (specifically, without nuclear membranes) and eukaryotic, those with a classical membrane-bounded nucleus. The model further posits that the former gave rise to the latter. The historical antecedents of this model are complex and rooted in the nineteenth century; for example, German biologist Ernst Haeckel positioned 'monera' (masses of protoplasm without a nucleus, later termed 'prokaryotes') at the base of his four-kingdoms phylogenetic tree.

The recognition that the main eukaryotic organelles, mitochondria and chloroplasts, were derived from bacteria by symbiosis between the bacteria and an ancestral eukaryotic cell prompted speculation on a similar origin for the eukaryotic nucleus. And the discovery of archaea — microbes that in many molecular ways resemble eukaryotes more than bacteria — resulted in proposals for archaeal origins for nuclear and cytoplasmic components of eukaryotic cells. Such proposals have sustained the concept that prokaryotes evolved into eukaryotes — an evolutionary model invoked by the terms themselves.

Molecular-sequence comparisons, first of ribosomal RNA genes in the late 1970s and of many other genes since, replaced analyses based on morphological subjectivities (such as the presence or absence of a nuclear membrane) with credible maps of evolutionary relationships between genes. These sequence comparisons have rendered the prokaryote/eukaryote model obsolete.

Ribosomal RNA, because of its ubiquity and slow rate of evolution, provides the most reliable view of the earliest evolutionary events. Comparisons of ribosomal RNA sequences show a three-domain tree of life (see figure). The diagram in essence is an experimentally derived map of biological organization and the course of evolution at the largest scale.



Comparisons of ribosomal RNA sequences reveal a three-domains tree of life, rendering the term 'prokaryote' obsolete.

Although some details of ribosomal RNA-based trees remain controversial, the basic three-domains structure and the relationships between the domains are generally accepted and are supported by observed biochemical variation. Phylogenetic trees based on all genes encoding the information-processing machinery needed to express genetic sequences are congruent with the three-domains tree. So the tree represents the evolutionary course of the genetic machinery, the functional core of genomes.

The lessons of the three-domains tree are profound. Instead of two kinds of organism, prokaryotes and eukaryotes, there are three: bacteria, eukarya (eukaryotes) and archaea. The root, or origin, of this universal tree, cannot be determined from ribosomal RNA sequences, but other phylogenetic results and biochemical correlates show that the genetic lines of eukarya and archaea have a common ancestral branch that is independent of that giving rise to the bacteria (see figure). That is, eukaryotes and archaea are more closely related to one another than either is to bacteria.

There is not a single (monophyletic) phylogenetic group upon which to hang the tag prokaryote. The major eukaryotic organelles, mitochondria and chloroplasts, are definitely bacterial in origin, but the nucleus is not. The nuclear line of descent is as ancient as the archaeal line and not derived from either archaea or bacteria. Thus, the prokaryote/eukaryote model for biological diversity and evolution is invalid.

Some have asserted that 'prokaryote' has utility, as a term for non-eukaryotes. However, this is a negative and therefore scientifically invalid description; no one can define what is a prokaryote, only what it is not. Lumping bacteria and archaea conceptually discounts fundamental differences between those two kinds of organism and reinforces an incorrect understanding of biological organization and evolution.

But if we can't call them prokaryotes, what should we call them? That, of course, depends on what is meant by 'them'. If what is meant is 'the little stuff out there', then try microbes or microbial.

Other things may need more precision. For instance, references in textbooks and in the literature to 'prokaryotic transcription' — meaning a transfer of information from DNA to RNA that relies on sigma transcription factors — overlook a deeper complexity. Archaea do transcription differently from bacteria, with TATA-binding proteins, much like those used by eukaryotes. Bacterial transcription is the correct term to use in this case. Another such oxymoron is 'prokaryotic protein synthesis', when the bacterial version is meant.

I believe it is critical to shake loose from the prokaryote/eukaryote concept. It is outdated, a guesswork solution to an articulation of biological diversity and an incorrect model for the course of evolution. Because it has long been used by all texts of biology, it is hard to stop using the word, prokaryote. But the next time you are inclined to do so, think what you teach your students: a wrong idea. ■

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FURTHER READING
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Gender: missing the prizes that can inspire a career

SIR — I congratulate Ben A. Barres on his excellent Commentary "Does gender matter?" (*Nature* **442,** 133–136; 2006). I was struck by the paucity of female plenary lecturers at the Bioscience 2006 meeting of the UK Biochemical Society. Spurred on by Barres's comment that too few women academics speak out against prejudice, I decided to do a little research on the matter.

There have been three meetings of the Biochemical Society in the new annual meeting format (Biosciences 2004, 2005 and 2006) and at these 1 of 10, 0 of 10 and 0 of 7, respectively, of the plenary lectures were given by a woman. Some of these plenary lecturers were recipients of prizes and medals, and I was so shocked by these statistics that I made a rough count of the proportion of women who have received these prizes over the years, as published on the society's website at www.biochemsoc.org.uk. Recipients' initials, rather than first names, are given, so I may conceivably have misattributed the male gender to some of the earlier names.

The prizes include the annual Colworth medal, given to a promising scientist under 35: only one has been awarded to a woman, out of 44 recipients, between 1963 and 2007. The statistics for the other prizes, up to 2007, are the Novartis medal, 2 of 39; Jubilee lecture, 1 of 23; Wellcome Trust award for research in biochemistry related to medicine, 1 of 11; AstraZeneca prize, 1 of 5; Frederick Gowland Hopkins memorial lecture, 0 of 24; Keilin memorial lecture, 0 of 21; Morton lecture, 0 of 14; Biochemical Society medal, 0 of 3; and GlaxoSmithKline medal, 0 of 2. This translates into 3.2% of the prizes being given to women, a truly lamentable record.

Furthermore, the statistics have not improved. In the past ten years, none of the Colworth medals has been awarded to women — and it is prizes such as these, given to scientists early in their career, that influence their future success. The results speak for themselves: that people will always give prizes to others in their own image, unless forced to take sexual and racial bias into account. I wonder if the record of other scientific societies is much better in this regard.

I should also point out that UK Biochemical Society meetings are supported by funds from the Biotechnology and Biological Sciences Research Council and by the European Molecular Biology Organization. Why do research funding bodies not assert leverage on this matter, by insisting that sexual and racial bias in speaker selection must be addressed at any meeting for which their financial support is given?

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Gender: macho language and other deterrents

SIR — In the Commentary article "Does gender matter?" (*Nature* **442,** 133–136; 2006), Ben A. Barres cites our article pointing out that the first round of the US National Institutes of Health (NIH) Pioneer awards was carried out in a way that would have predicted a bias against selection of women (M. Carnes *et al. J. Womens Health* **14,** 684–691; 2005). Indeed, no women were selected in the first year, so when 43% of the second year's winners were women we examined the process again to see what had changed.

We identified several differences, including changes made by the NIH, that would predict a decrease in the activation of automatic gender stereotypes that may have discouraged women from applying and disadvantaged women applicants in the first round.

First, a reduction in the number of applicants (from 1,300 to 840) and greater familiarity with an application process that was no longer new may have reduced time pressure on the reviewers.

Second, the NIH removed the repeated mention of the need for applicants to engage in 'high-risk' research; we believe that this terminology encouraged male and discouraged female applicants. Similarly, the emphasis on 'intrinsic' leadership abilities and 'potential' of the scientist was removed, in favour of an emphasis on the scientist's research.

Third, there was a much higher proportion of women in the applicant pool, which may have been related to the change in language (26% in phase 1 and 35% in phase 2 in 2005, compared with 20% and 10% in 2004). There was also a greater proportion of women on the review panel: 44% in 2005, compared with 6% in 2004.

Fourth, the presence of accomplished women scientists on the review committee provided a positive role model for applicants.

Finally, women were specifically encouraged to apply — a particularly significant factor in the context of the outcry in the scientific community following the absence of women in the first round.

We applaud the NIH for taking an evidence-based approach. Regardless of the gender composition of the group selected in the forthcoming third round, removal of conditions that are known to activate automatic gender stereotypes ensures that the best science will be supported, regardless of the sex of the scientist.

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See *Nature* 442, 510 (2006) for other letters on this topic. Readers are encouraged to add their comments on the Nature News Blog at: http://blogs.nature.com/news/blog/2006/07/does_gender_matter.html

A positive definition of prokaryotes

SIR — In his Concepts essay, Norman R. Pace argues that the concept of prokaryotes is misleading and proposes that the word 'prokaryote' be banned from the scientific literature[1]. We disagree.

Pace contends that the term prokaryote refers to the lack of a nucleus and that it is hence a "negative and therefore scientifically invalid description" of cell organization, because "no one can define what is a prokaryote". The former is a matter of opinion, and the latter is arguably incorrect.

Prokaryotes are cells with co-transcriptional translation on their main chromosomes; they translate nascent messenger RNAs into protein. The presence of this character distinguishes them from cells that possess a nucleus and do not translate nascent transcripts on their main chromosomes[2]. Although historically founded on a negative trait (lacking a nucleus), the term prokaryote does indeed specifically designate organisms that are defined by a positive character.

Pace proposes that we should speak only of archaea and bacteria instead of prokaryotes, and that if a collective term is needed to designate those cells that are not eukaryotes, the term 'microbe' should be used. That suggestion, too, is unacceptable, because many eukaryotes are microbes.

Regardless of what any gene tree might suggest and regardless of what anyone might believe about early evolution, modern cells lacking spliceosomal introns and spliceosomes[2], a nucleus, and mitochondria[3] do possess transcriptionally coupled translation — they are prokaryotes[4].

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RIKEN aids international structural genomics efforts

SIR — We would like to respond to comments made in your News story "'Big science' protein project under fire" (*Nature* **443,** 382; 2006) about Japan's Protein 3000 Project. This government project funds nine centres, including the RIKEN Structural Genomics/Proteomics Initiative, consisting of the RIKEN Genomic Sciences Center's Protein Research Group (http://protein.gsc.riken.jp) and the RIKEN SPring-8 Center.

First, we do not agree that the information gained is "of limited use" or, as one researcher is quoted as saying "a lot of it is junk". RIKEN has made major contributions to structures and structural models of functionally important proteins. It is expected to have determined 2,500 new structures by spring 2007, or 5% of the entire Protein Data Bank (PDB). Nearly half of those determined so far were obtained using NMR, and consist of functional domains from biologically important (including disease-related, signal transduction and nucleic-acid-binding) human/mouse proteins. Multiple structures from each family were analysed to understand binding specificities. About 70% of all NMR structures of human/mouse proteins deposited in the PDB in 2005 were from RIKEN.

The Protein 3000 project contributes significantly to the goals of the International Structural Genomics Organization (www.isgo.org) by providing large numbers of templates that can be used to model other members of the protein families. On average, each NMR structure from RIKEN has contributed to about 300 new homology models, and each X-ray structure to about 200 new models at a level of 30% sequence identity. Quality assessment measures for RIKEN structures are similar to those for structures deposited in the PDB in 2000–2006 by traditional structural biology groups, according to Gaetano Montelione of the Northeast Structural Genomics Consortium (personal communication).

Second, we very strongly disagree with the comment that "A centre of that size should contribute to methodology, but there has been nothing." RIKEN has made seminal contributions to the development of methodologies and technologies. RIKEN has pioneered cell-free protein synthesis on a production scale, and developed technologies for extensive sample optimization process using the cell-free method. These technologies have been indispensable in solving the structures of many difficult proteins. More than 1,000 NMR structures have been determined from protein samples synthesized by RIKEN's implementation of the cell-free method. Additionally, at RIKEN, N. Kobayashi has developed the KUJIRA software for spectral analysis and P. Güntert has developed the CYANA software for automated protein structure analysis. RIKEN will be opening the NMR facility, together with these important technologies, to external scientists in 2007.

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on behalf of the ISGO Executive Committee
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Advances in biology reveal truth about prokaryotes

SIR — Although we agree with William Martin and Eugene V. Koonin's point in Correspondence ("A positive definition of prokaryotes" *Nature* **442,** 868; 2006) about the validity of the term 'prokaryote', a term that Norman R. Pace has proposed abolishing ("Time for a change" *Nature* **441,** 289; 2006), they have lost sight of the organismic biology forest for the molecular biology trees. The main differences between prokaryotic and eukaryotic cells probably relate to the original symbioses from which eukaryotes evolved.

Eukaryotes — whether protoctists, fungi, animals or plants — routinely open their membranes to take in (or let out) nuclear genomes, whole cells or other large particles, in processes such as ingestion, fertilization and hybridization. They reseal their membranes and live happily ever after. All eukaryotic sexuality requires cell fusion. Nearly all eukaryotic cell phenomena involve microscopically visible intracellular motility that never happens in prokaryotes.

We need to reassess our understanding of the course of evolution by recognition of the differences between unidirectional transfer of genetic material as the basis of prokaryotic sexuality — genophore DNA, viruses, plasmids — and parental cell fusion in eukaryotes. Roger Stanier and Cornelius van Niel's concept of 'prokaryote' was brilliantly recognized in 1927 by Boris Kozo-Polyansky, who only wrote in Russian. The word 'procariotique' was independently coined in 1925 by Edouard Chatton for cyanobacteria and all other bacteria including archaebacteria (J. Sapp *International Microbiology* **9,** 163–172; 2006). Because of the modern developments of biochemistry and molecular biology, electron microscopy and comparative genetics, the term 'prokaryote' is even more valid now than it was when first introduced.

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Pollution analysis flawed by statistical model

SIR — I found your Special Report on air-pollution control in the United States ("The politics of breathing" *Nature* **444,** 248–249; 2006) to be generally well balanced. I would like to point out, however, that there is by no means universal agreement among scientists that air pollution at contemporary US levels affects human health. I am one of the sceptics.

The report seems to take at face value the conclusion of "two large, well-respected epidemiological studies", that every additional microgram of fine particles per cubic metre in the air causes tens of thousands of deaths a year in the United States. Yet joint pollutant analyses — with sulphur dioxide and either sulphates or fine particles both included in the statistical models — show that sulphur dioxide is associated with mortality; fine particles are not (D. Krewski *et al. Reanalysis of the Harvard Six Cities Study and the American Cancer Society Study of Particulate Air Pollution and Mortality* Health Effects Institute, 2002). The association of sulphur dioxide with mortality remains unexplained, as there is no plausible biological mechanism by which it could be causing death.

Further, the pollution studies mentioned used the proportional hazards model for analyses of the data. This model assumes that the relative risks of air pollution and potential confounders remain constant over time. It is clear, however, that the basic assumption of proportionality of hazards is satisfied neither for air pollution nor for a strong potential confounder, cigarette smoking (S. H. Moolgavkar *Inhal. Toxicol.* **18,** 93–94; 2006). Use of this model when the assumption of proportionality of hazards is violated can have serious consequences for the inferences drawn from the data. It may, for example, explain the very different results of observational epidemiological studies of hormone replacement therapy in the 1990s and the recently concluded Women's Health Initiative randomized trial (R. L. Prentice *et al. Am. J. Epidemiol.* **162,** 404–414; 2005). Departing from assumptions of proportionality of hazards for potential confounders may also bias the estimates of main effects in cohort studies, particularly when the confounder is a strong risk factor. In air-pollution studies, the use of a manifestly wrong model to adjust for confounding by smoking probably biases the estimates of small air-pollution effects on mortality, although the direction of the bias will depend upon the structure of the correlation between smoking and air pollution.

We do not currently have the methods to reliably estimate small environmental risks.

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