The Animal Tree of Life
Maximilian J. Telford
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second messenger signaling molecule cyclic adenosine monophosphate (cAMP).

To identify the enzyme generating cGAMP, Wu *et al*. and Sun *et al*. carried out three independent routes of purification of cytoplasm, each consisting of four steps of chromatography. Many proteins copurified with cGAS activity, but only three copurified in all three routes. One of the three is a member of the nucleotidyltransferase family, which includes adenylate cyclase, the enzyme that generates cAMP. This is especially interesting because cGAS would be predicted to be a cyclase on the basis of its amino acid sequence. The expression of endogenous cGAS was high in the screened cell line of the assay and in macrophages (immune cells that are critical for innate immunity) but very low in a cell line that does not contain an endogenous STING pathway. Among the many experiments carried out in both studies, the ectopic expression of cGAS and STING in the latter cell line fully restored responsiveness to DNA—an effect several orders of magnitude greater than that achieved by the ectopic expression of other DNA sensors such as DAI, IFI16, and DDX41. In vitro and in cells, DNA interacted directly with cGAS.

Wu *et al*. and Sun *et al*. provide compelling new insights into how DNA is sensed in the cytoplasm of mammalian cells. DNA binds to the enzyme cGAS, which catalyzes the production of the second messenger molecule cGAMP. This molecule in turn binds to STING, which triggers two different signaling cascades that launch the expression of host defense and inflammatory proteins (see the figure). Moreover, some bacteria appear to bypass cGAS by producing dicyclic nucleotides that bind to STING directly. The discovery of cGAS means that any microbe with DNA that stimulates gene expression by the transcription factors NF-κB and IRF3 will also signal via a cyclic dinucleotide, this time made by the host cell via cGAS. The pathway is also likely to be important for the sensing of self DNA, which can lead to autoimmunity.

What role does cGAS play relative to the other DNA sensors? This is not yet clear, and it is possible that cell type specificity will be found. Because cGAS has catalytic activity, it is possible that a small-molecule inhibitor could have therapeutic potential for autoimmune diseases. Whether that would leave the patient vulnerable to infection would need to be evaluated.

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EVOLUTION

The Animal Tree of Life

A molecular phylogeny of the animal kingdom published 25 years ago was the precursor to today's widely accepted phylogeny of all animal phyla.

Maximilian J. Telford

In a letter to T. H. Huxley written on 26 September 1857, Charles Darwin imagined a time to come "though I shall not live to see it, when we shall have very fairly true genealogical trees of each great kingdom of nature" (*1*). The publication of *On the Origin of Species*, two years later, prompted a century and a half of disagreement among zoologists proposing often wildly contradictory schemes of animal evolution. Clarity began to emerge with Field *et al*.'s landmark publication 25 years ago of an analysis of animal relationships based on ribosomal RNA (rRNA) sequences (*2*). The paper made zoologists realize that molecular biology could and should be applied to traditional zoological questions.

The earlier disagreements derived from varying interpretations of the morphological and embryological characteristics of animals. Many of these characters have evolved repeatedly in unrelated lineages as adaptations to similar selective pressures or have been lost from certain groups through disuse. Today's strengthening consensus is almost entirely thanks to the use of molecular genetic data in reconstructing trees. Heritable changes in nucleotides and amino acids are abundant and generally much less prone to the problems of convergent evolution and loss than are morphological characters (*3*).

Field *et al*.'s sequencing of 18*S* rRNAs from species across the animal kingdom narrowly predates the polymerase chain reaction (PCR) era (*4*). The authors instead produced their sequence data by direct reverse transcriptase sequencing of rRNA (*5*). They sequenced three regions of the 18*S* rRNA molecule from species representing 10 of the ~30 animal phyla. This approach produced ~1000 nucleotides of sequence per taxon, almost an order of magnitude greater than previous work using 5*S* rRNA (*6*).

If we consider a summary of the trees produced from these data (see the figure, panel A), we find some familiar groups (arthropods, chordates, and echinoderms), as well as some surprises. For example, almost all premolecular phylogenies supposed a close link between the brachiopods (lamp shells) and the deuterostomes (chordates and echinoderms). Yet in Field *et al*.'s tree, the brachiopods are placed far from the deuterostomes in the Lophotrochozoa, which include annelids and mollusks. This major rearrangement suggests that certain "deuterostomian" characters of brachiopods may have evolved more than once.

Similarly, premolecular phylogenies agreed on a close relationship of the annelids and arthropods (collectively the Articulata in reference to a body divided into segments that is typical of both groups). Field *et al*.'s tree provided the first hint that the annelids and arthropods are in fact independent groups, each more closely related to unsegmented phyla (see the figure, panel A).

Other surprises in the tree were less welcome. Probably the most striking result, and the one that provoked the strongest reaction at the time, was the conclusion that the multicellular animals evolved on two separate occasions from unicellular relatives (see the figure, panel A). It quickly became clear that this conclusion was incorrect and that it resulted from the cnidarians being

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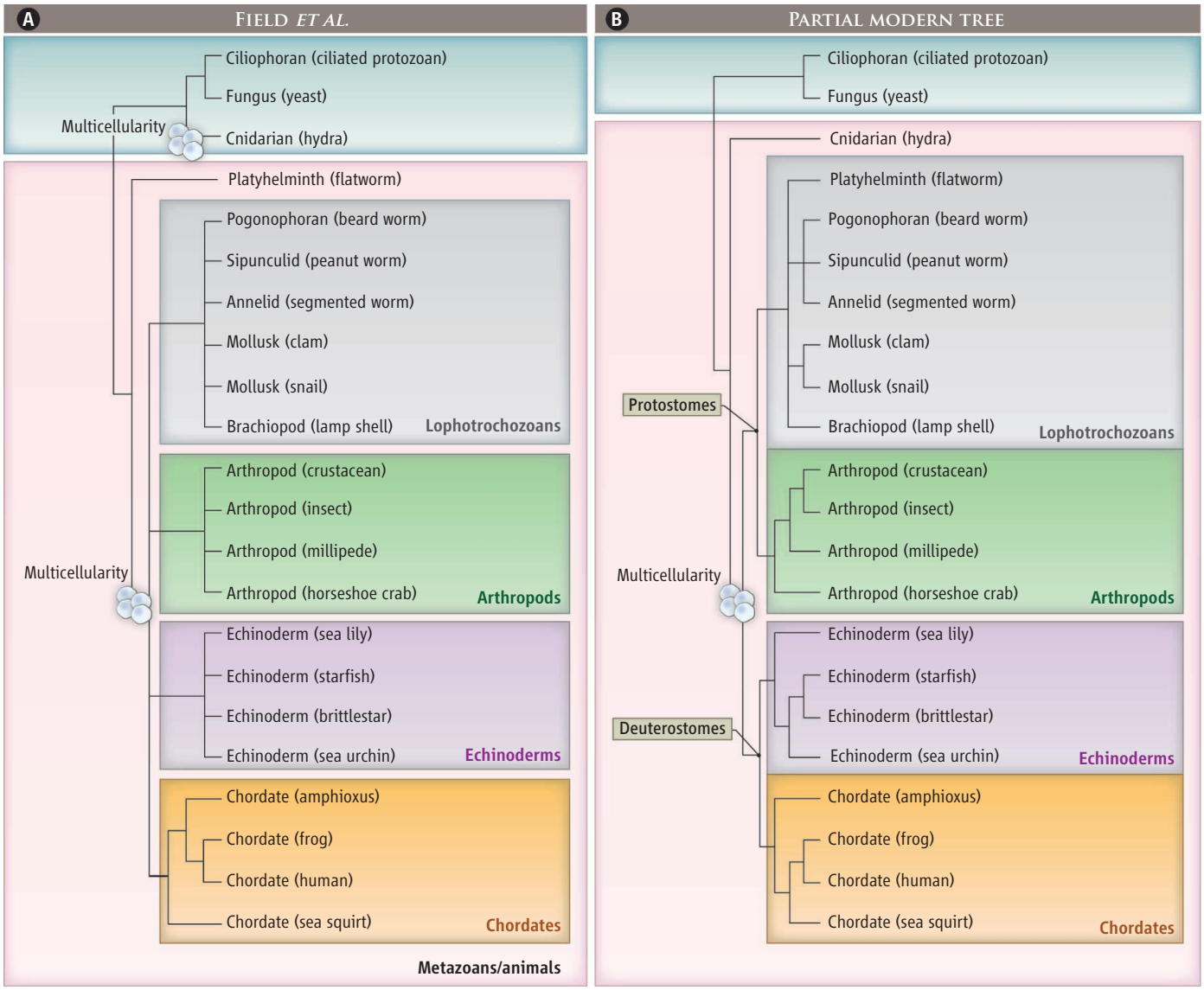

Toward a consensus. (**A**) Summary of the trees presented by Field *et al*. (*2*). This landmark tree began to clarify the evolutionary relationships of the animal phyla but could not resolve all relationships between groups at higher levels of the hierarchy. The tree contained some erroneous placements, the most striking of which was the conclusion that multicellularity evolved twice. (**B**) Modern tree. To facilitate comparison with (A), only those groups also studied by Field *et al*. are shown. Major groups are resolved into Protostomes and Deuterostomes. Today, constituent species of all 30 known animal phyla have been sampled. A more complete tree would, for example, include the pseudocoelomate phyla (including nematodes), which together with the arthropods constitutes the Ecdysozoa. The Deuterostomes contain two phyla (the hemichordates and xenacoelomorphs) that were not studied by Field *et al*. and are not shown.

misplaced in the tree. A second error—the placement of the flatworm *Dugesia* (Platyhelminthes) as a branch outside of the main groups of animals (see the figure, panel A)—took longer to resolve. We know now that its correct place is within the lophotrochozoans (see the figure, panel B) (*7*). Both errors arose because the 18*S* rRNA genes of the misplaced groups evolve at an unusually high rate, resulting in "long branch attraction," whereby rapidly evolving species are incorrectly placed close to the long branch leading to the species used to root the tree (such as yeast and ciliate, as in the trees in the figure) (*8*).

Building on the foundations of Field *et al*., some of the most important progress has stemmed from the development of probabilistic methods that can accommodate the systematic biases present in real sequences, such as unequal rates of evolution (*9*).

A second important trend has been an enormous expansion in taxonomic coverage. Field *et al*. covered 10 animal phyla; today, species from all 30 known phyla have been sampled (*10*). Broader sampling can help to improve the accuracy of the tree by allowing the experimenter to select among species from a given group to find those least affected by systematic biases and by highlighting systematic errors by providing information on the substitutions that have occurred along problematic branches. Both of these advantages of deeper sampling were instrumental in the identification of the Ecdysozoa, a group of animals that links the arthropods to other ecdysing (cuticle molting) animals such as nematodes (*11*, *12*).

The third important development has been the use of increasingly comprehensive multigene phylogenies. The earliest of these made use of large data sets derived from the first complete animal genomes to test the controversial Ecdysozoa grouping. The burgeoning availability of genome sequences

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from many phyla is now making the use of alignments of hundreds or even thousands of genes a standard procedure.

These studies have led to a widely accepted phylogeny of all animal phyla that has radically changed our views of animal evolution (*3*). Premolecular phylogenies generally envisaged a gradual increase in complexity from the earliest animals without a body cavity or coelom (acoelomate flatworms) via pseudocoelomate worms (such as nematodes and rotifers) to coelomate protostomes (annelids, arthropods, and mollusks) and deuterostomes (echinoderms and chordates) with a sophisticated mesoderm-lined coelomic body cavity.

In contrast, today's tree divides bilaterally symmetrical animals into protostomes and deuterostomes (see the figure, panel B). Within the deuterostomes, the simple urochordates (sea squirts) are closer relatives of the vertebrates than the more fishlike cephalochordates (amphioxus) (*13*); a third phylum of deuterostomes, the hemichordates (acorn worms), are the sister group of echinoderms and not of the chordates (*14*).

The acoelomate platyhelminths, as we have seen, are now known to be related to the coelomate annelids, mollusks, and brachiopods within the Lophotrochozoa. A second acoelomate group, the Xenacoelomorphs, although historically linked to the flatworms, have rather controversially been placed close to echinoderms to form a fourth phylum of deuterostome (*15*). Pseudocoelomate phyla, including nematodes and rotifers, are scattered throughout the protostomes.

All these rearrangements suggest that many characters thought to be important—such as the coelomic body cavity—have in fact been gained and lost multiple times.

Although much of the animal tree is now resolved, a number of problems remain. These problems tend to involve relationships either of taxa with extreme systematic biases or among groups that seem to have originated in a rapid radiation, resulting in a lack of signal supporting individual nodes. Future progress will depend on increasing useful signal with larger "phylogenomic" data sets from the widest possible taxonomic sample and on continued improvement in the correspondence between real data and the models used when reconstructing trees.

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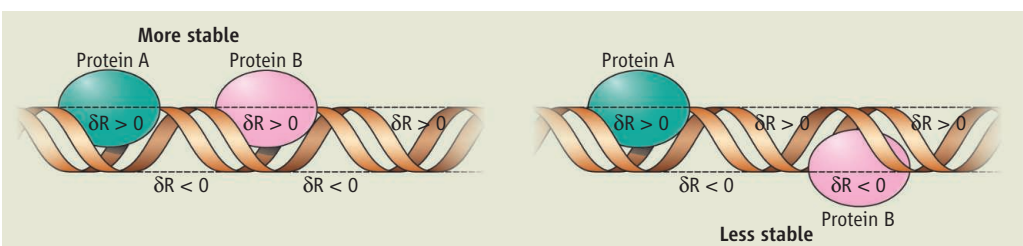
BIOPHYSICS

Fine Tuning Gene Regulation

An allosteric effect in which distortion of the DNA duplex by one protein modulates the binding of another protein may be important in gene regulation.

Donald M. Crothers

On page 816 of this issue, Kim *et al*. (*1*) report that a DNA-bound protein can influence the properties of an adjacent protein if both are bound to the DNA strand within about 15 base pairs (bp) of each other. The authors attribute their observations to an allosteric effect, in which a distortion of the DNA strand by the first protein modulates the binding of the second protein. The observations have important implications for gene regulation.



Allosteric coupling. Kim *et al*. show that oscillation of the major groove width $R(L)$ causes variation of the allosteric coupling between two DNA-binding proteins A and B, both of which widen the major groove. Thus, binding of A energetically favors binding of B at positions where R is already widened ($\delta R > 0$, top), but disfavors binding of B where R is narrowed ($\delta R < 0$, bottom). [Adapted from (*1*)]

The authors use single-molecule methods to detect the influence of the first protein (protein A) on the dissociation rate of the second protein (protein B), measured relative to the value without protein A. They show that the effect is strongly phase-dependent, with a periodicity of 10 bp and amplitude of ~4-fold change in the dissociation rate.

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The natural first interpretation of these results would be that the effect is due to protein-protein contacts or through-space electrostatic effects. However, these explanations are rendered unlikely by control experiments, which show that a hairpin loop can replace protein A, the effect is nearly independent of salt concentration, and the rate constant oscillation is much attenuated by a nick or unmatched base pair between the two proteins.

The authors studied various protein pairs, including the T7 RNA polymerase (T7 RNAp)–lac repressor (LacR) combination. In vitro single-molecule kinetic experiments showed that T7 RNAp stabilizes or destabilizes LacR, depending on the distance between them along the DNA strand. In transcription experiments in vivo, LacR was placed upstream of the T7 promoter used to transcribe the lac Z gene. It is a general thermodynamic principle that if one protein stabilizes/destabilizes the binding of another protein, the second must have the same stabilizing/destabilizing effect on the first. Lac Z expression levels, which