Cu-O spacing decreases). On the other hand Cohen and Falicov seem to assume that $|J_{pd}| \rightarrow \infty$ is possible, leading to $T_c \rightarrow \infty$.

Given that J_{pd} and J_{dd} are fairly tightly constrained, we assume that the variables that may be adjusted (by changing composition, structure, and so forth) to achieve the maximum T_c are λ (the strength of the coupling, which depends strongly on the concentration of holes in the Cu-O sheets) and τ [which depends on the distribution of Cu spins (magnons) for the system with migrating oxygen holes, $F(\omega)$].

There are two alternative approaches to increasing T_c: (i) increasing λ by increasing the number of holes on the oxygens in the copper-oxygen sheets (this is limited by the overall electrostatic energies that will tend to distribute the holes over the other atoms of the structure) or (ii) decreasing τ. [This requires modifying the distribution $F(\omega)$ to weight lower energy magnons. The migrating oxygen holes of the high T_c systems have the effect of doing this. However, we cannot yet calculate the $F(\omega)$ for this complicated dynamic spin system and thus do not have detailed suggestions on how to best decrease τ.]

Cohen and Falicov (*1*) also suggest that the cluster calculations of Guo, Langlois, and Goddard (*4*) lead to only rough estimates of the parameters. Since the T_c^{max} depends sensitively on J_{dd}, for which there is no direct experimental value (for the systems with Cu-O sheets), we carried out the same type of cluster calculation (generalized valance bond) on the K_2NiF_4 system (same structure as La_2CuO_4), where there are direct experimental values of $J_{dd} = -52$ K and -56 K (*5*). In this case the calculated value is $J_{dd} = -51$ K, which suggests that our values for the Cu-O system should be within about 20 K of the calculated 200 K.

There has been a recent experimental estimate made for J_{dd} of the Cu-O systems. Lyons (*6*), using Raman light scattering, found an inelastic peak at 0.37 eV for $La_2Cu_1O_4$ and 0.32 eV for $Y_1Ba_2Cu_3O_6$ (both semiconductors, not superconductors). As these systems are doped ($x > 0$), this peak rapidly disappears. They interpreted this inelastic transition as a double Cu spin-flip and deduced from linear magnon theory that $\Delta E = 5.4\ J_{dd}$, leading to $J_{dd} \sim -790$ K for 2-1-4 and $J_{dd} \sim -680$ K for 1-2-3. We believe that the large discrepancy with the calculated value argues against this interpretation. We suggest that for the undoped system there may be a small number of oxygen vacancies leading to extra electrons in the system, which would lead to local Cu^{I} (d^{10}) sites. From similar cluster calculations, we find that the excitation energy $Cu^{I}Cu^{II} \rightarrow Cu^{II}Cu^{I}$ near an oxygen vacancy is 0.4 eV and suggest that the Raman transition is associated with such $d^{10} - d^{9}$ interactions. For the 2-1-4 system, this could be tested directly by experiments at high O_2 pressure that would decrease the number of oxygen vacancy sites and by our suggestion lead to the disappearance of the 0.4-eV peak.

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Phylogeny and Molecular Data

Biologists with an interest in animal evolution have eagerly looked forward to the results of the new sequencing studies of genetic material, which many colleagues hope will provide "unequivocal phylogenetic trees." Such trees should once and for all solve the problems of homology versus analogy that have perplexed systematists for more than a century.

The recent article, "Molecular phylogeny of the animal kingdom," by Katharine G. Field *et al.* (*1*) reports the first results of a large investigation of ribosomal RNA from a number of phyla and illustrates the results with four evolutionary trees resulting from analyses of four slightly different selections of sequences.

Unfortunately, the four trees show four different branching relationships of echinoderms, annelids, arthropods, and chordates. The tree illustrating the more detailed relationships of some mollusks (a nudibranch, two clams, and a chiton), two annelids (a polychaete and an oligochaete), a pogonophoran, a sipunculid, and a brachiopod shows the brachiopod and the polychaete as sister groups derived from chitons and the earthworm as derived from another point within the mollusks. This will appear unacceptable to most systematists.

As the authors also state, analyses of additional portions of the RNA molecule will establish the branching orders with higher probability, but it is important to point out that the molecular data do *not* provide unequivocal phylogenetic trees and must be treated with just as much criticism, care, and tact as the traditional morphological characters.

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Perhaps the most striking and unexpected result of the investigation into metazoan phylogeny with 18*S* ribosomal RNA (rRNA) partial sequences, as reported by Field *et al.* (*1*), is the indication that the two sequences from Cnidaria (a hydra and a sea anemone) branch from a lineage including ciliates, fungi, and higher plants. They suggest that this provides strong evidence that the Cnidaria arose independently from other metazoan groups. However, they do not mention that this analysis contradicts the implication of 5*S* rRNA sequence data from a variety of Cnidaria. All the cnidarian 5*S* rRNA sequences clearly cluster with those of all other known metazoan 5*S* rRNA sequences, from a great variety of metazoans (*2*). The 5*S* rRNA sequence from a sponge also clearly clusters with that of metazoan sequences (*2*), although no 18*S* rRNA data from sponges are given by Field *et al.* On the basis of morphological simplicity, the relatedness of sponges to other metazoans has been more frequently questioned than that of Cnidaria (*3*). Even the 5*S* rRNA sequence from the primitively multicellular mesozoan *Dicyema misakiense* suggests probable branching from the metazoan lineage at an early stage (*4, 5*). The 5*S* and 18*S* rRNA data are in agreement in suggesting that the sequences from the planarian *Dugesia* represent the most isolated metazoan lineage (*1, 2*). The admittedly incomplete and controversial fossil record suggests a nearly simultaneous initial radiation of lineages representing Cnidaria and a variety of other metazoans, 600 to 700 million years ago (*6*). This is consistent with the 5*S* rRNA

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Response: The writers have raised several interesting issues concerning methodology and phylogenetic interpretation of ribosomal RNA (rRNA) sequence data and concerning our unexpected finding that the 18*S* rRNA sequences of coelenterates (Cnidaria) suggests that these creatures may have arisen from protists independently of other animals.

Nielsen is disturbed by the placement of organisms in some of the trees. Specifically, he questions the four different branching orders for eucoelomate phyla shown by the trees, and he is disturbed by the implied sister group relationships in the detailed tree for protostomes. Both of these concerns stem from overly detailed interpretation of the trees. As we discussed in our paper, a slowly evolving molecule is required to resolve very old phylogenetic events. However, during a rapid radiation of lineages, a slowly evolving molecule may accumulate too few mutations in the interval of interest to resolve the splitting events. In this case, rapid radiations will appear as a polychotomy without topological definition: perhaps the tree should have been drawn as such. However, we felt it better to present the quantitative trees rather than an interpretive sketch. As we pointed out in the paper, we in fact do not suggest any branching relationships for echinoderms, protostomes, chordates, and arthropods from our data. Instead, we have shown that molecular data support the concept of the explosive late Precambrian radiation of animals that is suggested from paleontological data. The same considerations apply to the protostome tree. As above, the topology of these closely spaced branch points cannot be resolved. What is clearly shown by the data is that there was a complex radiation of protostome groups soon after the radiation of coelomate animals. Further data will be needed to resolve the branching orders. Additional interesting results have come from the analyses of protostomes. First, there is indeed a complex of closely related protostomous phyla. Second, brachiopods are in fact closely related to the classic protostomes. Third, mollusks are close to annelids. The molecular data clearly make untenable the commonly held view that mollusks are independently derived from platyhelminthes (*1*). It is in discriminating between models like these that the rRNA sequence data make a major contribution. We agree with Nielsen that molecular data are no panacea for systematics. Rather, such data are an inevitable part of modern systematics because they offer new lines of evidence. Moreover, the sequence data provide the most incisive basis for comparison in some instances: what other phylogenetically informative homologies are there between mammals and clams and corn?

We concur with Walker that the 5*S* and 18*S* rRNA-derived phylogenies are in significant disagreement. Of the Metazoa for which both 5*S* and 18*S* rRNA data are available, the anemone, starfish, and sea urchin sequences display the greatest inconsistencies. If these two molecules are not consistent with a common phylogeny, then is one of the phylogenies more acceptable in the light of other data? In the case of the echinoderms the answer is clear: the 5*S* data suggest a polyphyletic origin of echinoderms, whereas the 18*S* rRNA data group them together very solidly. For anemone and hydra, the 18*S* rRNA data place the origin of Cnidaria near the plant-fungal separation, whereas the 5*S* rRNA data place jellyfish and anemone within the Metazoa, but within the Bilateria instead of as a sister group of the Bilateria. Certainly the latter placement is biologically unreasonable. The question is whether the former placement is also unacceptable.

After the publication of our paper, we reexamined the statistical significance of the affiliation of Cnidaria with the plants, fungi, and ciliates by construction of phylogenetic trees from our cnidarian sequence data using the "bootstrap" method of Felsenstein (*2*). In this procedure trees are constructed on the basis of a random sampling (with replacement) of sequence positions. When the cnidarian sequences are sampled in this way, 54% of the resultant trees reproduced the published grouping of taxa. The next most common outcome (42%) is the positioning of the Cnidaria as a sister group to the Bilateria. In 50 resamplings of the data we never observed divergence of the Cnidaria before the separation of the ciliate and fungal lineages from that leading to Bilateria, or after the branching of the flatworm lineage from that giving rise to the coelomates.

Walker further suggests that a minor 18*S* rRNA gene could have become dominant in the cnidarian lineage. If the common ancestor of the Cnidaria and Bilateria had multiple, distinct 18S rRNA genes and if different versions were preserved in the two subsequent lines of descent, then an inappropriate phylogeny might be inferred—equivalent to comparing alpha globins of some mammals with beta globins of others. Although variation among the 5*S* rRNA genes within an organism has been observed in various metazoans (*3*), it is only recently that Gunderson and his co-workers (*4*) discovered that the protist *Plasmodium berghei* expresses two different 18*S* rRNA genes during its life cycle. However, there is no evidence that such occurences are common, although they have been looked for in many systems; and the 18*S* rRNA data remain consistent with metazoan monophyly. Perhaps the search for inconsistencies should turn to asking why the 5*S* rRNA yields such implausible evolutionary relationships [for examples see (*5*)].

Bode and Steele also question the position of Cnidaria derived from 18*S* rRNA data, but on different grounds. They point not to conflicts between sequence-derived inferences, but to a variety of persuasive cellular, subcellular, and molecular features that Cnidaria share with other animals. However, before rejecting an independent origin for these groups from the protists, we should consider that an independently evolved "animal" would have many of the same features as true metazoans. Protists possess many of the precusors for the parallel evolution of such structures as muscle and nervous tissue. The close resemblance of these structures in cnidarians and other animals could reflect ancestry, or it could reflect the constraints of such systems regardless of origin. The same arguments apply to subcellular structures. Protists are both incredibly diverse and unfortunately little studied at the molecular level. As Bode and Steele point out, Cnidaria possess some molecular features (for example the *src* gene) in common with other metazoa that have not to date been identified in protists, yeast, or plants. The 70% sequence similarity between hydra and chicken *src* genes speaks to a very high degree of evolutionary conservation in this sequence, and it may indeed prove to be very useful for tracing long-distance phylogenetic relationships. However, the presence or absence of a specific molecule may not be a definitive character. To illustrate, the *ras* oncogene is present in both yeast and slime mold and it is 65%

sequence data.

How are we to explain this clear discrepancy in apparent phylogenetic origin of cnidarian 5*S* and 18*S* rRNA sequences? The 18*S* sequences presumably should be more reliable indicators of organismic phylogenetic relationships, on the basis of a much greater number of nucleotides. However, as Field *et al.* emphasize, extreme differences in rates of nucleotide substitutions can obscure phylogenetic origins. There is no suggestion that the 5*S* rRNA cnidarian sequences diverged at a notably faster or slower rate than did the mean metazoan sequence (7). Figure 2 in the 18*S* analysis (*1*) indicates unusually short branches for the two cnidarian sequences relative to their presumed time of divergence (*8*). However, it is not clear how this might result in a major error in branching assignment for the cnidarian sequences. Typically there are many 18*S* and 5*S* rRNA genes in metazoans, as well as in other eukaryotes. Thus, the possibility presents itself that a relic minor 18*S* rRNA genotype was carried in the early metazoan lineage and became dominant in the cnidarian lineage. Alternatively, we might propose that the smaller 5*S* rRNA sequences of protocnidarian and protometazoan lineages converged. This would seem a fortuitous chance event. Could selection have been involved? Could lateral gene transfer between protocnidarian and protometazoan lineages or protocnidarian and protistan lineages have occurred? Until the apparent discrepancy between the 5*S* and 18*S* rRNA sequence analyses is resolved by data from other molecules or perhaps by a more illuminating phylogenetic analysis methodology, the significance of the finding that the 18*S* rRNA sequence data do not support a common phylogenetic origin of Cnidaria with other metazoans should be treated with caution.

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We read with interest the article by Katharine G. Field *et al.* (*1*). The authors constructed a phylogeny based on the comparison of the 18*S* ribosomal RNA sequence of a number of organisms. Particularly interesting is the conclusion that metazoans arose twice and independently from an early protistan ancestor. Cnidarians are inferred to have arisen from a branch that gave rise to fungi, ciliates, and plants, whereas the rest of the animals are said to have arisen from a separate branch. However, a number of other cellular, subcellular, and molecular characteristics that cnidarians share with other metazoans, but not with plants, fungi, or ciliates, render this conclusion unlikely.

Nervous systems composed of neurons are unique to animals. The nervous system of cnidarians, while not as complex as that of other metazoans, is made up of cells that are easily identifiable as neurons. In hydra, they have the typical morphology of interneurons or sensory cells (*2*). The ultrastructure of the chemical synapses formed by these neurons with other neurons and with the muscle processes of epithelio-muscular cells is similar to those found in many other animals (*3, 4*). The sensory cilium-stereociliary complex formed in sensory cells is also similar to that seen in vertebrate hair cells (*4*). The neurons express neuropeptides found in many metazoans [see, for example, (*5*)]. Additionally, a neuropeptide originally identified in hydra (*6*) is expressed in the nervous tissue of several mammals [see, for example, (*7*)].

Cnidarians have several subcellular structures with complex organization in common with other animals. As is typical of invertebrates, the occluding junctions between epithelial cells of, for example, hydra and Polyorchis, are septate junctions (*8, 9*). The ultrastructure of these junctions is identical to that in many invertebrates, as is their occurrence in circumferential bands around the apical ends of the epithelial cells.

Epithelial cells are also connected to one another by gap junctions that have the same ultrastructural features observed in many animals [see, for example, (*8, 9*)]. Further, they appear to have a similar function. Small fluorescent dyes (for example, Lucifer yellow), but not large ones (for example, fluoresceinated dextran) will pass from one epithelial cell to the next (*10*), which is typical of gap junction–mediated cell-to-cell communication. An antibody raised against rat liver gap junction protein recognizes a protein in hydra with the same molecular weight as that of the gap junction protein in many species of animals (*10*). When introduced into cells, the antibody specifically interrupts communication between epithelial cells (*10*). In hydra (*11*) and other cnidarians (*8, 12*), gap junctions have been observed between neurons and between neurons and muscle processes of the epithelial cells.

Even though there are no separate muscle cells in cnidarians, epithelial cells contain muscle processes in which the contractile elements are arranged in a manner identical to that in other metazoans. The circumferential swimming muscles of many medusae have the appearance of typical striated muscle found in other animals. The myofibrils exhibit the usual arrangement of sarcomeres with A-bands, I-bands, and Z-lines [see, for example, (*12, 13*)]. In hydra, the myofibrils of the epitheliomuscular cells have an arrangement similar to that of smooth muscle (*14*).

In addition to the molecules mentioned above that cnidarians share with other metazoans, two more are associated with animals but not with other organisms. Between the two epithelia of every cnidarian is a mesoglea, a basement membrane. The mesoglea of several cnidarians has been shown to contain collagen [see, for example, (*15*)]. Further, in the two instances where it has been analyzed, the number of glycosylated hydroxylysine residues is high, as is typical of collagens found in basement membranes (*15, 16*). Finally, it has been shown that all metazoans including cnidarians contain a tyrosine kinase that is immunoprecipitable by pp60^{src} antibodies (*17, 18*). Such a kinase is absent from all plants and unicellular organisms, including ciliates, examined (*17*). Recently, the *src* gene of hydra has been sequenced and found to have 65% homology with the *src* gene of chickens at the amino acid level (*19*).

Because the Cnidaria share these many characteristics with other metazoans, but not with fungi, ciliates, and plants, it is difficult to accept the proposed biphyletic origin of the cnidarians and the rest of the animals. The amount of convergent evolution that would have been required to explain the shared characteristics seems improbable. Thus, we feel the preponderance of the evidence indicates that it is more likely that the Cnidaria arose with the other metazoans, as is traditionally described.

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