zoologists) groups, the Lophotrochozoa (*6*) (combining Eutrochozoa and Lophophorata) and the Ecdysozoa (*1*) (moulting animals). The basal status of the acoelomates and the pseudocoelomates is vanishing in view of this new evidence: Most of the acoelomates do not show an early emerging position but are found in the Lophotrochozoa together with coelomate phyla (*1*, *7–9*). The pseudocoelomates, or aschelminths, do not form a monophyletic group: Rotifers and gastrotriches are grouped with the lophotrochozoans (*8*), whereas kinorhynchs, priapulids, nematodes, and nematomorphs are found with the arthropods in the Ecdysozoa (*1*, *8*). The traditional phylogeny is thus turned upside down. The bilaterian tree is divided in three great coelomate lineages (see figure), among which the simply organized acoelomates and pseudocoelomates are scattered. It is quite comforting that data of a completely different type, namely, the evolution of the *Hox* gene cluster, appear to confirm some aspects of the above phylogeny, such as the affinities of flatworms (*9*).

Another remarkable feature of these trees is the consistently poor resolution of the relationships within these three superphyla, as illustrated by the failure to retrieve the monophyly of well-recognized phyla such as annelids, molluscs, and arthropods (*1*, *10*). This absence of resolution may positively indicate a rapid process of radiation that occurred at the beginning of the history of each of these branches (*11*). This corresponds well with the paleontological data, as the earliest unambiguous fossil representatives of the superphyla all appear suddenly in the Lower Cambrian [mollusks and brachiopods (*12*) as lophotrochozoans, arthropods and priapulids as ecdysozoans, echinoderms (*13*) and chordates (*14*) as deuterostomes].

This remarkable trifurcation of the Bilateria leads us to a new interpretation of the Cambrian explosion as simultaneous radiations of three long-separated stem lineages. The lineages diverged well back in the Precambrian but were poorly diversified before the Cambrian. Attempts to date the protostome-deuterostome divergence with several types of molecules have given conflicting dates of either more than one billion years (*15*) or just 670 million years (*16*). These calculations nevertheless agree on the existence of a large fossil gap between the separation of the three stem branches and their actual appearance in the fossil record. It has been suggested that this gap may be due to the fact that the Vendian ancestors of the large coelomates were tiny animals, unlikely to give fossils (*17*). This hypothesis supposes that a major event allowed the size of metazoans to increase dramatically at the beginning of the Cambrian. However, large-sized metazoans were already present during the Vendian. We think that the three-branched bilaterian phylogeny also strongly contradicts this view. If these tiny ancestral worms had existed before the Cambrian, their hypothetical, unchanged, simply organized descendants (platyhelminths, nematodes, gastrotriches, rotifers, and so forth) would root back to a "basal" position in the bilaterian tree, forming a "phylogenetic lawn" in which the three coelomate branches would be rooted. Instead, acoelomates and pseudocoelomates are found dispersed among the coelomate phyla in the two protostomian branches. Thus, they are likely to be derived, secondarily simplified forms. We suggest that the ancestors of the three coelomate branches may have been macroscopic coelomate animals. In that case, these macroscopic animals being rare or restricted to environments unlikely to allow fossilization may explain their absence in the Precambrian fossil record. However, their already complex body plans would have constituted the necessary preadaptations for their explosive diversification in the Cambrian.

Should this hypothesis of three simultaneous radiative events be accepted, it would also argue in favor of an external cause for the Cambrian explosion. One cannot resort to a single internal genetic mechanism (such as the "invention" of the *Hox* cluster) to explain the radiation of three long-separated lineages. An ecological type of explanation [such as the rapid building up of new complex trophic networks in the context of an ecological vacuum (*18*)] should thus be investigated.

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CHEMISTRY

Rethinking Solution NMR

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In the 50 years since publication of the first solution nuclear magnetic resonance (NMR) data, NMR has evolved from explorations of esoteric quantum mechanical properties (nuclear angular momenta and dipole moments) into what is by far the most powerful form of spectroscopy employed by the practicing chemist. Part of the reason for this evolution is the maturation of radio frequency technology: Staggeringly complex manipulations with radio frequency pulses and pulsed field gradients are trivial to implement. Much more importantly, however, the theoretical framework of NMR is mature and tractable. Researchers can calculate how the magnetization evolves under such complex manipulations and can optimize pulse sequences to extract structural information. Even undergraduate students can readily analyze one- and two-dimensional solution spectra of moderately sized molecules (with, for example, 100 hydrogen atoms), and complete analyses of the spectra of proteins with molecular masses of order 25 kD have become commonplace. Nonetheless, one of the most intriguing modern research directions in NMR is the rethinking of the assumptions behind its "standard picture." This rethinking, as reported for example by Glaser *et al.* (*1*) on page 421 of this issue, is leading toward substantially improved pulse sequences, permitting measurements of entirely new parameters, and extending the range of sample sizes and molecular sizes accessible by NMR.

One might argue that NMR spectra should be exceedingly difficult to interpret. Imagine that we could detect the signal from a single

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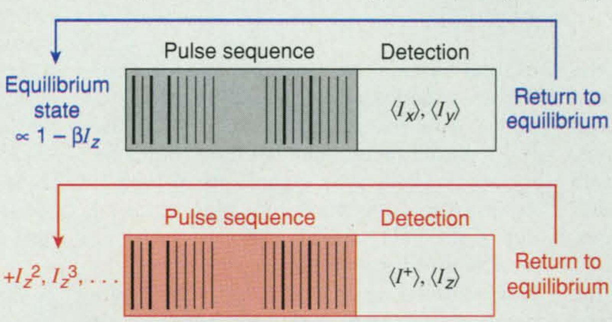
100-spin molecule in our room temperature sample. Forgetting everything but the possible nuclear spin states, each of these molecules has $2^{100} \approx 10^{30}$ different possible states. In a very large magnet, the energy difference between spin states is on the order of 10^{-4} kT. Hence, the molecules are distributed between a vast number of different states. Each state has a completely different NMR spectrum, with peak amplitudes and phases varying wildly and erratically from the "normal" spectrum we describe to undergraduates. Multidimensional experiments should be almost impossible, because relaxation takes the molecule to a completely different state with each pulse sequence repetition. In addition, each molecule rotates and vibrates in solution and interacts with the field generated by magnetic dipoles on every other molecule. The net effect is tens of kilohertz of resonance frequency variation on each spin, fluctuating on a picosecond time scale. From this perspective, how could we hope to understand a solution NMR spectrum, which requires >1 s of spin evolution?

The solution to this conundrum—an elegant and brilliantly successful hybrid of quantum mechanics and statistical mechanics—evolved over the first few decades of NMR research (figure, top). During the time before exciting or detecting the signal (blue in the figure), the system is described by statistical mechanics. This works because in a typical experiment the signal might come from 10^{18} molecules in the active region of the sample tube. More explicitly, we use the Boltzmann distribution to find an initial equilibrium density matrix ρ_{eq} for an "average molecule" and then retain only the identity matrix (unimportant in the evolution) and a small initial magnetization along the magnetic field axis. We also allow the system to always relax back to this same state.



Two views of NMR. (**Top**) Standard picture in which the Hamiltonian *H* is independent of spin density matrix ρ, time evolution is linear, oscillating magnetization is detected, and molecules are independent. (**Bottom**) New approach where *H* depends on ρ, evolution is nonlinear, non-Hermitian operators or forces are detected, and cooperative interactions between molecules occur.

On the other hand, during the excitation and detection (gray and black in the figure), we describe the evolution using almost pure quantum mechanics. Rapid isotropic tumbling and diffusion are assumed to eliminate all intermolecular couplings. The electronic, vibrational, and orientational degrees of freedom are treated completely separately from the nuclear spin states (in NMR parlance, we separate "spin space" from physical space). This creates an apparent Hamiltonian in which only chemical shifts, scalar couplings, and external fields affect nuclear spin operators. Finally, we assume that we can continuously observe a pair of noncommuting Hermitian operators (the transverse magnetizations I_x and I_y) without perturbing the system. With these assumptions, the equation describing the evolution of our "average molecule" looks identical to Schrödinger's equation—except perhaps for exponential decays imposed to account for slow relaxation and cross-relaxation. Time evolution of the system is described by a linear equation and is represented by unitary transformations. The Hamiltonian is independent of the spin state, and it becomes possible to fully understand and predict the spin evolution.

Many of the assumptions in this standard picture are now being reworked (figure, bottom). For example, Glaser *et al.* (*1*) point out that the detected operators I_x and I_y are generally combined into a single complex parameter $I_x + iI_y$ (where *i* is $\sqrt{-1}$), which in turn is the (non-Hermitian) raising operator I^+. The authors then ask the deceptively simple but quite important practical question: Given any possible unitary transformation, how does one maximize the transfer of the desired signal (for example, a specific coherence) into this non-Hermitian operator? The authors predict substantial improvements over existing sequences that were designed by merely asking the seemingly more sensible question of how to maximize I_x and I_y.

My group and others have been investigating cooperative interactions between molecules in solution (*2*). Dipolar couplings between distant molecules (>10 μm) are not averaged away by diffusion. This leads either to a classical picture that requires nonlinear equations of motion and propagators that depend explicitly on the state of the system or to a quantum picture that starts from higher order terms in ρ_{eq} and predicts observable signals from intermolecular multiple-quantum coherences. The experimental signals are large (often >10% of the full equilibrium magnetization). Demonstrated applications include improving contrast in magnetic resonance imaging and removing inhomogeneities without removing chemical shifts.

David Cory and co-workers at the Massachusetts Institute of Technology have been exploring excitation sequences that include massive field gradients (as large as 10^5 G/cm). These gradients can actually generate a measurable phase difference between coupled spins (*3*), which must generate a nonunitary transformation on the reduced density matrix, where all intermolecular degrees of freedom have been traced away. Sidles, Yannoni, and co-workers at IBM have demonstrated potential advantages of detecting the actual force exerted by nuclear spins in a magnet (proportional to I_z) rather than the electromagnetic field induced in a coil (*4*). Weitekamp and co-workers at Caltech, using dimensional arguments, have shown that this approach may remain useful even as we approach the limit at which there are so few spins that the uncertain component of the angular momentum ($\sqrt{N}$ h/2 for *N* spins) is larger than the angular momentum from the equilibrium magnetization (*5*). That group has also recently reexamined the normal treatment of NMR in dynamic systems. They have found previously unsuspected terms in the spin Hamiltonian, which might lead to substantial errors in previously published equilibrium constants or average structures (*6*). Finally, Bax and co-workers at the National Institutes of Health have shown that dissolving a small concentration of bicelles in a protein-water solution creates a slight preferred protein orientation (order parameter about 10^{-4}). This reintroduces small dipolar couplings and shifts the resonances of directly coupled nuclei (such as ^{15}N-^{1}H), thus defining the direction of the internuclear vector and aiding structural determinations (*7*).

In most of these experiments, the underlying physics is not new: For example, dipolar couplings in oriented solutions and the dipolar demagnetizing field in isotropic solution were first observed decades ago, and force detection dates back to the earliest days of NMR. Rather, these experiments extend the boundaries of NMR with an approach enunciated most clearly by Schrödinger himself: "Thus, the task is, not so much to see what no one has yet seen, but to think what nobody has yet thought, about that which everybody sees" (8).

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