

Frischmeyer *et al.* (2) and van Hoof *et al.* (3) demonstrate that nonstop mRNA decay in both *S. cerevisiae* and mammalian cells requires neither the major deadenylase nor players in either the 5'-to-3' or NMD pathways. Instead it involves cytoplasmic exosome-mediated decay that begins at the 3'-poly(A) tail. The protein Ski7p associates with the cytoplasmic form of the exosome and with an auxiliary complex of Ski proteins (Ski2p, Ski3p, and Ski8p) through its amino-terminal domain (12). The completely or partially codon-free A site of the leading ribosome that is stalled at the 3' end of the poly(A) tail is recognized by the carboxyl-terminal domain of Ski7p. This domain closely resembles the GTPase domains of the translation factors EF1A and eRF3 (13), which interact with the ribosomal A site occupied by a sense codon and a nonsense codon, respectively. An exosome mutation that inhibits Ski7p from interacting with the exosome also inhibits nonstop mRNA decay. Thus, van Hoof and co-workers (3) propose that the amino-terminal domain of Ski7p recruits both the exosome and the Ski complex to the 3' end of nonstop mRNAs (see the figure).

Frischmeyer and co-workers (2) justify

the need for nonstop mRNA decay by citing evidence that 40 of 3266 expressed sequence tags in *S. cerevisiae* (1.2%) derive from mRNAs having a 3' end located upstream of the termination codon. They also show that 52 of 6357 translational reading frames in *S. cerevisiae* (0.8%) and 239 of 32,755 translational reading frames in humans (0.7%) harbor a putative 3'-end processing signal. In theory, nonstop mRNAs can also be generated when 3'-end formation takes place upstream of the normal termination codon as a result of RNA polymerase pausing (which is a known contributor to 3'-end formation), when ribosomes pause at rare codons or normal termination codons (as they do to trigger tmRNA function in bacteria), or when 3'-to-5' decay is initiated on ribosome-bound mRNA.

One of a number of issues that remain to be resolved is whether eukaryotic nonstop mRNA decay involves a protein that mimics tRNA, similar to eRF1 [which functions with eRF3 in nonsense codon recognition (14)] or a tRNA-like RNA. Furthermore, the fate of the protein products of nonstop mRNAs remains a mystery. Molecular tags for proteolysis in eukaryotes usually consist

of multiubiquitin chains that target the protein for degradation by the 26S proteasome (15). Because proteases responsible for the degradation of the protein products of nonstop mRNAs in bacteria are related to components of the proteasome (15), it is conceivable that the protein products of nonstop mRNAs in eukaryotes are ubiquitinated and then degraded by the proteasome. How the degradation of nonstop—and, for that matter, nonsense—mRNAs and the proteins they produce are coordinated is an area for future study.

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PERSPECTIVES: CHEMICAL REACTIONS

Steric and Solvent Effects in Ionic Reactions

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Noncovalent interactions play a central role in chemical structure and reactivity. Chemical reaction rates are influenced strongly by nonbonding interactions, particularly the solvation processes that evolve as reactants approach, and the short-range repulsions that constrain motion through the transition state. But separating the effects of solvation from the repulsive interactions (the steric effect) has challenged chemists for over a century (1–3).

On page 2245 of this issue, Regan *et al.* (4) take an important step toward resolving this problem. They report gas phase rate measurements and computer simulations on a classic nucleophilic substitution (S_N2) reaction. The results provide quantitative estimates of the distinct contributions of the solvation and steric effects to the free energy of activation in this important organic reaction.

In an S_N2 reaction, an incoming reactant X^- (the “nucleophile”) approaches a saturated carbon atom in a “backside attack” (see the figure). As the nucleophile approaches the central carbon atom and the incipient C-X bond forms, the umbrella associated with the tetrahedral geometry about the carbon atom begins to invert. The noncovalent interactions that influence motion through the transition state are very sensitive to the size of substituents R_1 , R_2 , and R_3 bound to the reaction center (5, 6). The rates of nucleophilic substitution in alkyl halides decrease by several orders of magnitude as the alkyl substituents increase in size.

The study of chemical reactions such as the S_N2 reaction in the gas phase is a valuable approach for probing their dynamics without the complexities of solvent interactions (5, 6). According to the double minimum potential model for the gas phase S_N2 reaction (see the figure) (7), the reaction proceeds through an electrostatically bound reactant-like complex $X^-\cdots RY$, which rearranges to a product-like

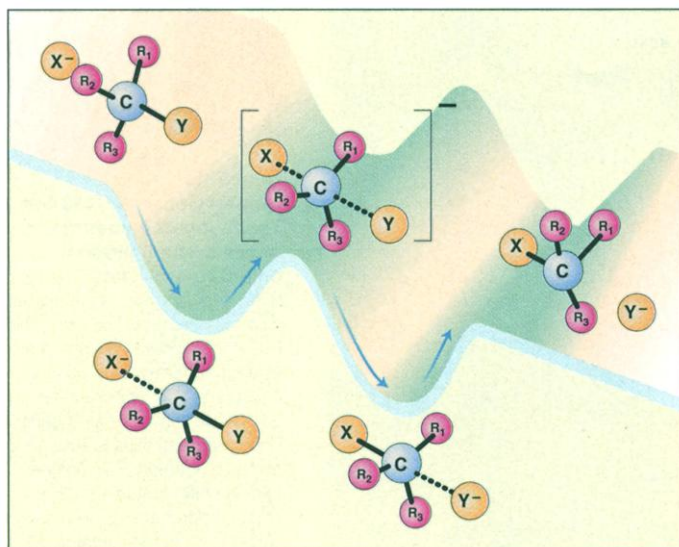
complex $RX\cdots Y^-$. The latter intermediate then decays to reaction products.

Experimental studies that isolate the precursor complexes for both the forward and reverse processes and probe their reactions (8–10) demonstrate that these complexes are true reactive intermediates on the S_N2 reaction coordinate. The barrier that separates these complexes provides a direct measure of the steric effect in the reaction, after correction for differences in the bond strengths of the forming and breaking bonds.

Even though the steric barrier in the gas phase lies below the energies of the approaching reactants and therefore does not manifest itself directly as a reaction threshold, it serves as an entropic bottleneck to product formation. Once the reactant-like complex forms, it can proceed through the sterically hindered transition state and cross the barrier to form products, or it can revert to reactants. The calculated branching ratio, evaluated with Rice-Ramsperger-Kassel-Marcus (RRKM) statistical rate theory (11), is a parametric function of the steric barrier height. The best estimate of the barrier height is the value that reproduces the experimentally observed rate. A significant body of experimental and theoretical research (12) confirms the validity of the statistical hypothesis in the present system.

Regan *et al.*'s careful experiments provide a direct measure of the steric barrier.

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Schematic reaction coordinate for the S_N2 reaction. Repulsions between the R_1 , R_2 , and R_3 substituents in the transition state are responsible for the steric effect.

Unlike previous studies that made use of less sensitive experimental methods and were constrained to probe faster, exothermic reactions, Regan *et al.* examine an “identity” reaction, that is, the nucleophile and the leaving group are identical. This approach eliminates the need to remove the contribution of the reaction exothermicity to the measured steric barrier (13).

Identity reactions proceed at rates that are at most a few percent of the encounter-controlled rates characteristic of gas phase ion-molecule reactions. Fourier transform

mass spectrometry methods that allow the necessary long observation times are therefore essential for reliable rate measurements.

Regan *et al.* show that the intrinsic barrier height for chloride exchange in 2-chloro-3,3-dimethylbutyronitrile is 1.6 kcal/mol higher than in 2-chloropropionitrile. The magnitude of this intrinsic barrier increase (attributable to the increased steric effect as *t*-butyl is substituted for methyl on the reactive carbon center) is about one-quarter of the barrier increase observed in aqueous solution.

The authors augment this experimental determination with molecular dynamics simulations that evaluate the contribution of solvation effects to the free energy of activation. The calculations show that substitution of *t*-butyl for methyl at the reactive carbon results in a free-energy increase of 4 kcal/mol at the transition state. The total increase in the barrier height, 1.6 kcal/mol from the intrinsic barrier and 4 kcal/mol from solvation, is in good agree-

ment with the 5- to 7-kcal/mol increase observed in aqueous solution.

The study provides a fundamental benchmark for quantitative understanding of the critical contributions that noncovalent interactions make to chemical reaction rates. The clear separation of functional group substitution into an intrinsic steric component and a contribution from solvation will lead to a deeper understanding of the fundamental relations among chemical structure, dynamics, and reactivity. The ability to optimize chemical reactions, develop new chemistry, and predict the properties of complex chemical systems will benefit all disciplines that work at atomic and molecular length scales.

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PERSPECTIVES: COSMOLOGY

The Beginning of Time

Craig J. Hogan

The Cosmic Background Explorer (COBE) satellite (1) scanned the farthest reaches of the sky for 4 years to assemble data for its historic map of primordial radiation. The blotchy pattern in the

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map (see the figure), which represents tiny variations in radiation brightness, is caused by small-amplitude perturbations in the structure of space-time that stretch across billions of light years of space today; they are the largest structures we will ever be able to see.

It now seems likely that these structures may also be magnified images of the smallest

things we will ever be able to see. The pattern is a faithful image of quantum fields—individual elementary particles whose imprint was frozen into the fabric of space-time very early and was then stretched to enormous size by cosmic expansion. Similar fluctuations led to the gravitational formation of all astronomical structure we see today, including our own Galaxy and its stars and planets.

In the last 2 years, experiments on high-altitude balloons circling Antarctica and in the high desert passes of the Andes have returned low-noise maps of smaller patches of sky than COBE, but with much finer angular detail than COBE's blurry 7° beam. A NASA spacecraft, the Microwave Anisotropy Probe (MAP), is now gathering data and should, within a year, return a detailed all-sky map at better than 1° resolution. The European PLANCK mission aims

to create an even more detailed map with even higher accuracy a few years later.

The closer studies of the perturbations now under way are yielding precise measurements of the main global parameters of the universe and may provide concrete data on the long-sought connections between quantum mechanics and gravity (or the fields of mass-energy and the space-time within which they act).

The biggest and the smallest things in nature are connected by a set of ideas called inflationary cosmology (2–5). Physicists postulate that the dynamics of the early universe are controlled by a new field called the inflaton, whose behavior resembles that of the Higgs field, a central component of the Standard Model of particles and interactions. (The Higgs boson, although not yet directly observed, is responsible for the masses of elementary particles such as the electron, and some of its properties are already quite precisely known.)

For both fields, gravity can have the remarkable property of being repulsive rather than attractive. If the properties of the infla-

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