The mechanistic scheme in the first figure thus appears to apply also to this class of monoiron enzymes.

Lastly, Ninian Blackburn (Oregon Graduate Institute of Science and Technology, Beaverton, OR) elaborated on a new mechanism for oxygen activation by the dicopper enzymes peptidylglycine α-amidating monooxygenase (PAM) and dopamine β-monooxygenase (DBM). Crystallography has shown that the two copper centers of PAM are separated by 11 Å in a solvent-accessible cavity (*17*), a gap too large for efficient electron transfer needed to generate a putative Cu(II)-OOH oxidant. The crystallographers suggested that the substrate occupies this cavity and serves as a "wire" between the two copper centers, but Blackburn's spectroscopic studies suggest that the energetic cost of the required reorganization would limit the efficiency of electron transfer. Instead, he has proposed a novel superoxide channeling mechanism (*18*), where O_2 is first reduced to superoxide at one copper and then channels through the solvent-filled cavity where it reacts with the second copper to form the hydroperoxo intermediate. This mechanism, in which superoxide carries both the electron and the coupled proton, represents a new paradigm for monooxygenation reactions at copper centers in proteins.

The symposium stimulated lively discussions among the participants and emphasized the richness of the chemistry associated with oxygen activation at metal centers. A diverse set of techniques has been used to probe the behavior of enzymes and model systems, and much progress has been made, particularly in our understanding of heme enzymes. Future challenges lie in elucidating the corresponding mechanistic details in nonheme enzymes and determing what adaptation Nature has devised to facilitate oxygen activation at such active sites.

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1. The 2000 International Chemical Congress of Pacific Basin Societies (Pacifichem 2000) was held in Honolulu, Hawaii, from 14 to 19 December 2000.
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PERSPECTIVES: RNA STRUCTURE

Pulling on Hair(pins)

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A broad range of single-molecule techniques now permit direct observation of the activity of single ion channels (*1*), protein and RNA enzymes (*2*, *3*), molecular motors (*4*, *5*), and even larger macromolecular assemblies (*6*). A shared characteristic of all these single-molecule observations is a thermally driven all-or-none discrete transition between conformations. We have come to think that this all-or-none behavior depends on the molecule's having a very large number of interacting atoms, which generate highly cooperative conformational changes. In this issue of *Science* (page 733), Liphardt and colleagues have discovered that short RNA hairpins placed under a stretching force undergo all-or-none discrete transitions in length, which follow a time course strikingly similar to those of transitions observed in ion channels and enzymes (*7*) (see the figure).

RNA is more than a mere messenger: It can fold into three-dimensional structures—ribozymes—that are capable of enzymatic activity (*8*). The self-assembly of RNA enzymes is simpler than that of proteins. For example, in proteins, most secondary structures depend on the global amino acid sequence and are not independently stable, whereas RNAs assemble in a hierarchical manner. Secondary structures such as hairpins, bulges, and three-helix junctions form quickly into stable entities. Once formed, these structures begin a slow dance in search of the final tertiary contacts. These hierarchies make the study of RNA folding a more tractable problem than protein folding. Nevertheless, the folding landscape of RNA enzymes is a complex collection of multiple pathways and transient states that would be difficult to discern with bulk studies. Single-molecule studies, on the other hand, can follow the individual time trajectories of folding and unfolding dynamics.

The work of Liphardt *et al.* elegantly demonstrates these advantages. These investigators used optical tweezers to apply a small force to individual segments of RNA secondary structures suspended between two polystyrene beads. The laser tweezers trap the beads and stretch the RNA structures with a force that can be finely controlled in the piconewton range. Using this approach, they probed the folding of "a simple RNA hairpin, a molecule containing the three-helix junction, and the P5abc domain of the *Tetrahymena* ribozyme" (*7*). The study of the stability of these structures along a well-defined reaction coordinate, the end-to-end distance of the molecules, illuminates how RNA makes possible the formation of structures with proteinlike qualities.

For example, under a stretching force of 14 pN, the RNA hairpin undergoes rapid cycles of all-or-none extension-contraction events of ~18 nm. If the force is increased by only a fraction of a piconewton, the hairpin remains in the extended form, and a small reduction in the force causes the hairpin to remain folded. All these effects can be simply described by assuming that the applied force does mechanical work on the hairpin and changes the height of the activation-energy barrier for the unfolding/folding reaction, as described initially by Bell (*9*). At a critical force, when the unfolding and folding rates are equal (for example, 14 pN), the hairpin is observed to spend equal time in each state. Furthermore, dwell times were found to be exponentially distributed, indicating that the conformational transitions lacked memory (a Markovian process).

The Markovian all-or-none kinetics and the steep force dependence of the dwell times observed for a simple RNA hairpin are hallmarks of ion-channel kinetics. What is going on here? Similarly to the effect of a force, the membrane electric field does work on the ion-channel structure ($W = zeV$), altering the height of the activation-energy barrier and changing the rates exponentially with the applied voltage. At a given membrane potential, the opening and closure rates become equal, and the channel is observed to spend equal times in both states. Hence, the mechanisms generating the kinetics and voltage dependence of an ion channel are very similar to those of a hairpin under a stretching force (see the figure).

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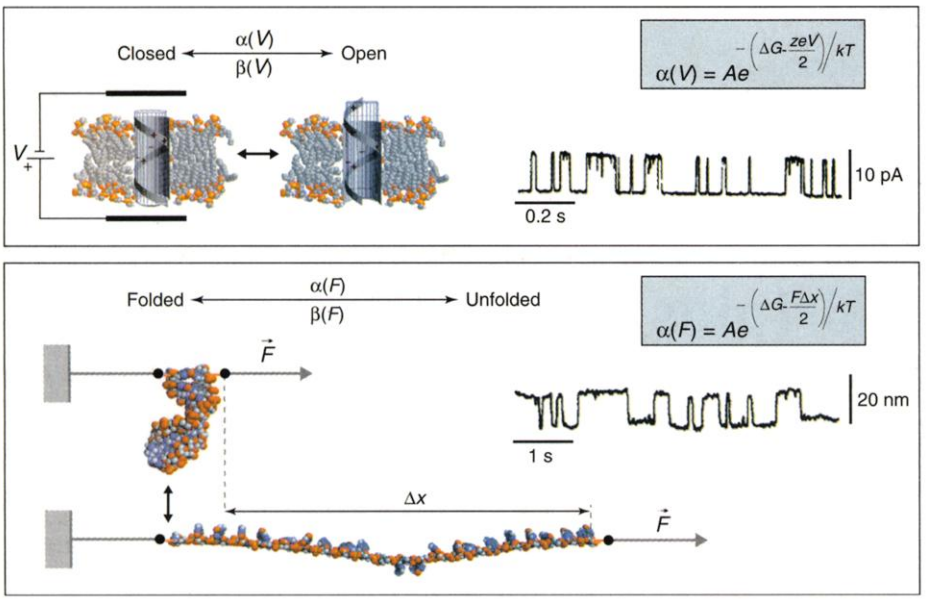
This analogy also carries a warning. The early recordings of ion-channel activity revealed little more than simple two-state kinetics. However, improvement in the time resolution of the recording apparatus later revealed a plethora of additional conformations with rapid kinetics that greatly increased the complexity required to describe these proteins. Similarly, we may find that an improved time resolution brings a much greater complexity to the kinetic behavior of the RNA hairpins.

The hopping folding kinetics of the RNA hairpin is surprising. Given its simple structure, we expect a hairpin to extend gradually owing to sequential unzipping that may have been observed in short DNA constructs (*10*). What sort of magic gives rise to the all-or-none kinetics of the RNA hairpin? A possible explanation is that the hairpin is more than the sum of its bonds, and that in forming a structure (helix), it becomes very sensitive to small perturbations. This is also seen in the mechanical unfolding of small proteins that, despite a complex tertiary structure and pattern of hydrogen bonding, also mechanically unfold with two-state kinetics (*11*).

A simple two-state kinetic description of the hairpin folding reaction does not clarify the physics underlying this reaction. Typically, we think of stable conformations as the result of minimizing the energy of the atomic interactions of a molecule. This is easy to imagine when a molecule is tightly coiled and a conformation is acquired by a relatively small rearrangement. However, it is difficult to picture what sorts of interatomic interactions remain when a hairpin is mechanically unfolded and stretched (see the figure). Yet, under such conditions, the hairpin is indeed able to spontaneously contract to its folded native state, doing work against the optical tweezers and the DNA handles. Is this picture plausible? Not really! To understand this point we must consider some basic elements of polymer elasticity. Polymer chains oppose stretching, because elongation reduces their degrees of freedom, and the resulting reduction in the entropy of the chain costs mechanical work. Hence, the relaxed state of a polymer chain is coiled. For any applied force, there is an equilibrium extension of the polymer, which can be calculated from several models of polymer elasticity. We used the wormlike chain model of polymer elasticity to calculate that at 14 pN, the unfolded RNA hairpin will extend to 75% of its contour length. However, this represents only an average extension, whereas the polymer is undergoing thermally driven fluctuations in length that may cause a nucleating event that leads to the cooperative folding of the hairpin. In addition to its spontaneous thermal fluctuations, the hairpin is tethered to the beads of the optical trap through semi-rigid DNA-RNA handles. Hence, the fluctuations that drive the folding reaction arise not only from the RNA polymer, but also from the fluctuations of the beads and those of the handles. For example, we calculate that a trapped bead with a spring constant of 0.1 to 0.03 pN/nm will fluctuate, on average, by ~6 to 12 nm, which is comparable to the length of the reaction coordinate. The authors recognize these limitations and incorporate a correction into their rate constants. However, we still cannot be certain of the influence of the apparatus on the actual kinetics of the hairpin. Answering this question will require further experimentation.

The remarkable kinetic properties of the RNA hairpins observed by single-molecule techniques open the possibility that similar experiments will be done soon on the hairpins and other autonomous folding units of proteins (*12*). Protein hairpins have already been shown to fold with two-state behavior and are thought to capture most of the basic physics of protein folding (*13*, *14*). Similar to RNA (*15*), protein hairpins are thought to nucleate folding (*16*). Future single-molecule experiments may indeed demonstrate two-state Markovian kinetics for isolated protein hairpins. If the observed hairpin folding rates are found to be similar to those of the parent protein, it may be tempting to conclude that hairpins not only can nucleate folding, but also function as pacemakers of the folding reaction.



Single–ion channel and–RNA hairpin unfolding kinetics. The opening and closure of an ion channel by an electric field is a highly cooperative event, leading to all-or-none fluctuations between the closed and open states (**upper panel**). The equation in the inset relates the opening rate constant α to the energy required to open the channel, where ΔG is the height of the activation energy barrier at zero voltage, e is the elementary charge, and z is the number of gating charges that move in the electric field. The trace on the right illustrates a typical recording of the activity of a single ion channel. The all-or-none unfolding of an RNA hairpin (**lower panel**) can be triggered by a mechanical stretching force, F, applied to the 3′ and 5′ ends. A mechanical force increases the probability of unfolding by exponentially speeding up the rate of unfolding α and decreasing the refolding rate β. The trace on the right shows a recording of the all-or-none changes in length from a single RNA hairpin [from (*7*)].

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