pected to solve the protein-folding problem by brute force.

The computation of atomic forces during a binding or unbinding process has more powerful applications than comparison with a rupture experiment. Forces indicate bottlenecks in binding pathways and hot spots in molecular conformations. Forces in the direction of an artificial constraint, averaged over local equilibria, are equivalent to the derivative of free energy profiles along the constraint and allow the reconstruction of such free energy profiles. Careful analysis of the pulling force, if the process is slow enough to ensure intermediate equilibration, should yield the free energy of ligand binding. The biotin-streptavidin binding free energy has already been determined from simulations by free energy perturbation techniques (*6*), but the pulling force method also predicts the probable path. If the inverse (binding rather than unbinding) can be modeled, pulling toward the binding site can provide automatic docking procedures with quantitative estimates of thermodynamic binding properties.

The use of average forces in a constraint direction to derive free energy profiles has been demonstrated in a completely different application: the prediction of water permeability through a lipid membrane (*7*). The solubility of water in a lipid membrane is so small that water permeability cannot be studied directly in MD simulations. But by constructing the free energy profile of water across the membrane and determining local diffusion constants, one can accurately compute the permeability. The free energy profile is integrated from the average force acting on water molecules constrained at a given depth in the membrane. In this way, MD simulations on a nanosecond scale can predict processes that occur on much longer time scales.

Biomolecular dynamics has come of age for two reasons. First, computers now allow routine nanosecond-scale simulations for systems of biologically relevant size, and thus, one can obtain at least local equilibrium and derive thermodynamic and transport characteristics. Second, methods have now become available to permit extrapolation to longer time scales and larger system sizes. In the near future, the link must be established between detailed MD and dynamical descriptions on coarser time and length scales, such as mesoscopic dynamics (*8*). This link will provide the "ab initio" understanding of macroscopic phenomena. The article by Grubmüller *et al.* is a milestone on this exciting path.

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# When Proteins Receive Deadly Messages at Birth

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According to Mayan tradition, the first human beings turned out badly and the gods Tzakól, the Creator, and Bitól, the Former, quickly destroyed their creations. Since those days, quality control pathways have evolved to work at the molecular level, and now life cannot function properly without them. Proteins with errors can either be killed or cured: They are eliminated by proteolytic enzymes or repaired by molecular chaperones. Aberrant polypeptides expressed from defective mRNAs, however, are irreparable, and for such proteins, destruction is the only option. In this issue of *Science* (*1*), Keiler *et al.* describe a stunning new quality control mechanism for proteins in bacterial cells that serves precisely this function. By invoking a previously unknown ribosomal translation mechanism, nascent polypeptides, translated from truncated mRNAs lacking stop codons, receive short COOH-terminal peptide tags that target the resulting polypeptide fusions for degradation.

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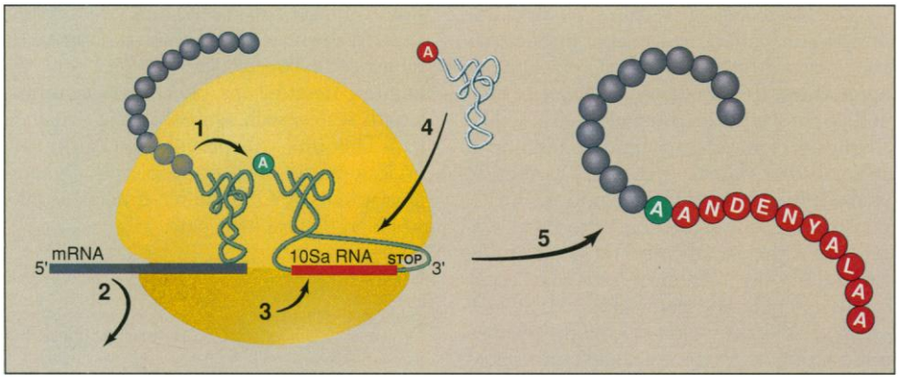
This story emerged last year when Tu *et al.* (*2*) reported that a fraction of mouse interleukin-6 (IL-6) protein expressed in *Escherichia coli* received a novel COOH-terminal modification. These variants were truncated at the COOH-terminus to a different extent but contained an identical COOH-terminal peptide tag with the sequence AANDENYALAA, which is not encoded by the IL-6 mRNA. This new sequence, except for the first alanine, is encoded by a small (362 nucleotides) stable RNA, known as 10Sa RNA and expressed from the *ssrA* gene. A portion of 10Sa RNA can fold into a tRNA-like shape and can be charged with alanine (*3*). Thus, Tu *et al.* suggested that the added peptide tag is derived from this alanine residue and the translation product of 10 codons from the 10Sa RNA.

Sauer and co-workers realized that the added COOH-terminal tag resembled the degradation signal of an enzyme called tail-specific protease (Tsp; also known as Prc) (*4*) studied by their group. This enzyme, localized in the periplasm of *E. coli* cells, binds certain apolar COOH-terminal tails of substrates and cleaves proteins endoproteolytically (*5*). A cytosolic counterpart of the periplasmic Tsp protease, which also degrades proteins in a tail-dependent manner, appears to exist in *E. coli* cells, although it has not yet been characterized (*6*). Keiler *et al.* (*1*) inserted transcriptional terminators into the reading frames of one periplasmic and one cytosolic protein and observed that the resulting partial transcripts lacking stop codons were indeed translated with a COOH-terminal tag encoded by the 10Sa RNA. Remarkably, the periplasmic tagged fusion protein was degraded by Tsp, and the cytosolic protein by Tsp's cytosolic counterpart.

What is the biological relevance of this unusual tagging mechanism? Because of the action of nucleases, mRNAs may be truncated and lose their encoded stop codons at their 3′ ends. Translation of those messages can still proceed but comes to a halt when the ribosome reaches the 3′ end of the mRNA. In the absence of a stop codon, release factor cannot trigger the dissociation of nascent polypeptides from ribosomes, and all ribosomes engaged in translation of the same reading frame are stalled. Alanine-charged 10Sa RNA may come to the rescue (see figure). 10Sa RNA appears to combine both transfer and messenger RNA properties (I therefore suggest it be called a tmRNA). Keiler *et al.* (*1*) argue that the tRNA-like domain enables the ribosome to catalyze the next peptidyl transfer of the nascent chain to the 10Sa RNA–bound alanine. The defective mRNA can then be released, and the ribosome switches to the reading frame provided by the 10Sa RNA.




**Marking the mistakes.** When an mRNA lacking a stop codon is made in error, translation is stalled and the incomplete polypeptide chain remains bound to a tRNA. Alanine-charged 10Sa RNA binds to the ribosome, and peptidyl transfer of the polypeptide chain to the 10Sa RNA–bound alanine (green) occurs (step 1). After mRNA release (step 2), the ribosome switches messages (step 3), and a short open reading frame on the 10Sa RNA (shown in red), encoding the peptide ANDENYALAA, is translated by charged tRNAs (step 4) until the ribosome reaches a stop codon. This process releases the polypeptide with a COOH-terminal tag (step 5), which is recognized by cytosolic and periplasmic tail-specific proteases. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; L, Leu; N, Asn; and Y, Tyr.

This process, message switching, might resemble frameshifting or ribosomal hopping (*7*), with the significant difference that it works in trans, as the new reading frame is provided by a second molecule. Charged tRNAs complementary to the codons of 10Sa RNA are then used for translating the encoded tag. The tagged polypeptide can finally be released as the ribosome reaches the in-frame stop codon of the 10Sa RNA.

This novel translational trick is particularly appealing, as it kills two birds with one stone: It not only clears the ribosome of defective messages, but also tags and thereby eliminates potentially harmful peptide products. Because tail-specific proteases exist both in the cytosol and the periplasmic space, this pathway can destroy defective translation products released from ribosomes to the cytosol or, after translocation, to the periplasm. Tsp is an adenosine triphosphate–independent protease with limited ability to attack stably folded proteins (*5*). Unstructured, tagged polypeptides are, however, probably good substrates for this enzyme. Tsp can also act on native proteins as a COOH-terminal–processing enzyme: Penicillin-binding protein–3 of *E. coli* and the photosystem D1 protein from cyanobacteria are COOH-terminally processed by Tsp and its homolog, respectively (*4*, *8*). Both substrates appear to display Tsp-recognizable sequences at their COOH-termini, which are encoded by their own mRNAs.

Tsp and 10Sa RNA have homologs in both Gram-positive and Gram-negative bacteria (*1*, *8*). Whether eukaryotes possess an analogous system is not known; this pathway might be restricted to organisms where transcription and translation are tightly coupled. Tagging proteins for degradation, however, is a common theme in eukaryotes and prokaryotes, although previously known degradation tags are not attached translationally. In the eukaryotic ubiquitin/proteasome system, proteins receive ubiquitin tags (a 76-residue polypeptide) at lysine side chains, and conjugates are subsequently degraded by the proteasome (*9*). Further, by a tRNA-mediated process, certain proteins in both eukaryotes and prokaryotes are tagged at the $NH_2$-terminus with single amino acids that target the modified protein for degradation by the N-end rule pathway (*10*).

This novel tagging pathway might also possess regulatory functions. Indeed, 10Sa RNA mutants not only exhibit slow growth but also have such specific defects as reduced motility, delayed recovery from carbon starvation, and aberrant phage growth (*3*, *11*). Truncated mRNAs may arise accidentally but may, in some instances, actually be desirable. Retroregulation, a controlled directed 3′ to 5′ digestion of polycistronic mRNAs, is one example (*12*). Designing tmRNAs encoding biochemical tags could identify regulatory proteins subject to this translational tagging. Nature could even utilize "trans-translation" more widely for generating protein fusions expressed from two different RNAs. The future will reveal what other surprises the ribosome has in store.

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