m ents of gas-phase species used as tracers (39) indicate that the layers had been transported from the tropics. Such layered profiles are typical of the mid-latitude stratosphere in fall, winter, and spring (our summertime data are too few to allow general interpretation). Other analyses of tracer measurements have shown that quasi-horizontal transport from the near-tropopause region of the tropics occurs rapidly in fall, winter, and spring to the mid-latitudes between the tropopause and altitudes of at least 19 km (38, 40). Together, these observations indicate that particles produced in the upper tropical troposphere are frequently transported to the lower mid-latitude stratosphere, where they may significantly affect particle number and surface area mixing ratios.

### REFERENCES AND NOTES

11. O. B. Toon, personal communication.
26. Mid-latitude aerosol characteristics were taken from the MAESA. Data from STEP 87 were taken from flights to, and from Panama were used from the AAOE data set. The SPACED flights were based from Moffett Field, California, and the ASHCE and MAESA flights were made from Moffett Field to Christchurch, New Zealand, with transit flights to Hawaii and Fiji. Measurements within the polar vortices have been excluded from this data set.
33. K. H. Rosenlof, personal communication.
36. Mid-latitude aerosol characteristics were taken from a profile measured on 29 December 1988 (5).
39. S. C. Kiang and D. D. Davis, Global Biogeochem. Cycles 7, 15889 (1993). The contributions of K. H. Rosenlof, C. M. Volk, P. J. Sheridan, K. A. Boering, F. Gelbard, and O. B. Toon are gratefully acknowledged. Data were collected under several grants from the National Aeronautics and Space Administration (NASA); analysis was supported by NASA grant NCC 2-792.
40. J. C. Wilson et al., personal communication.
We conducted experiments using an assay developed (6, 7) to study transcriptional elongation by single molecules of E. coli RNA polymerase in vitro (10). Ternary transcription complexes consisting of single molecules of RNA polymerase associated with a DNA template and a nascent RNA transcript were assembled in solution, halted by NTP depletion, and adsorbed onto the cover glass surface of a microscope flow cell. Polystyrene beads (0.52 μm in diameter) were attached to the transcriptionally downstream ends of the DNA molecules so that each bead became tethered to the surface by its connection through the DNA and the polymerase. When supplied with NTPs, up to half of the immobilized transcription complexes are enzymatically active; bead-labeled complexes display elongation kinetics indistinguishable from those of unlabelled complexes in solution (6, 7).

In a typical experiment, a transcription complex was located by video-enhanced, differential interference contrast light microscopy. The complex was identified visually by the Brownian motion of the bead, which was constrained by the length of its DNA tether to a small region centered over the attachment position of the polymerase (6, 7). For the studies described here, the microscope was equipped with an optical trapping interferometer ("optical tweezers" plus a position sensor) that could exert calibrated forces up to ~100 pN on a bead while simultaneously measuring its displacement with subnanometer precision and millisecond time resolution (9, 11). With the laser light shuttered, the optical trap was moved to the region near the bead. The shutter was then opened to activate the trap, and the bead was captured. By adjusting the trap controls, we positioned the center of the bead directly over the polymerase, typically at a height of ~590 nm above the cover glass surface (this particular height was chosen to bring the bead as close as possible to the surface without the risk of touching during subsequent measurements). We then repositioned the trap by moving it at a constant height along the direction of greatest detector sensitivity [the direction defined by the shear axis of the Wollaston prism; see (9, 12)] until the DNA straightened and was held under very light tension, with the bead displaced just 30 to 70 nm from the trap center (Fig. 1A).

We started transcriptional elongation of bead-labeled complexes by exchanging the buffer inside the flow cell with one containing NTPs. In most experiments, elongation was begun immediately before establishment of the initial configuration just described. During elongation, the template was pulled by the stationary polymerase molecule, developing further tension in the DNA between the bead and the polymerase and drawing the bead away from the trap center (Fig. 1B). The optical trap acts as a nearly linear spring of stiffness αtrap attached to a stationary reference frame and exerts a force Ftrap on the bead (Fig. 1C).

The bead adopts a position where Ftrap is balanced by the force Ftc exerted by the polymerase, acting through the DNA. The series elasticity due to the DNA, polymerase, and associated linkages acts as a spring of stiffness αDNA. By calibrating the optical trap stiffness and determining the displacement of the bead, we could measure Ftrap and thus Ftc. The force could be determined without knowledge of the stiffness αDNA, which depends on the length of DNA as well as the applied force (13).

During control experiments in which no NTPs were added, beads remained at an approximately fixed distance from the trap center. Slight changes seen in the interferometer signal (mean rate ~0.3 nm s⁻¹ toward the bead) were attributable to instrumental drift.

In elongation experiments using high concentrations of NTPs (1 mM each of adenosine triphosphate, guanosine triphosphate, cytidine 5'-triphosphate, and uridine 5'-triphosphate), 1 μM PPi, and low trapping force (laser power at specimen, 25 mW; αtrap ~0.03 pN nm⁻¹) (14), we observed continuous movement of beads out to the limit of the usable range of the trap, located roughly 200 nm from the trap center. Once a bead reached this limit, the trap was manually repositioned to bring the bead closer to the trap center so that observation could continue (Fig. 2A). In five of seven elongating complexes studied in this way, beads moved continuously to the limit without stopping, moving at similar velocities after the trap was repositioned. (One complex stopped and failed to restart; another stopped for ~18 s and then continued elongation to the trap limit.) The bead velocity, 4.3 ± 1.3 nm s⁻¹ (mean ± SD) (15), was comparable to elongation rates measured under similar conditions in solution (4.4 to 6.8 nm s⁻¹) (6) and in previous microscope experiments (4.2 ± 1.7 nm s⁻¹) (7).

Different behavior was observed when higher trapping forces were used (laser power at specimen, 82 to 107 mW; αtrap ~0.09 to 0.12 pN nm⁻¹). In this regime, 66 of 77 elongating complexes (86%) stopped translocating (stalled) once the bead encountered the high-force region of the trap (Fig. 2, B and C). To test whether stalling was reversible, we maintained the bead at stall, typically for 10 to 15 s, then repositioned the trap center closer to the bead, reducing Ftrap. After force reduction, 24 of the 66 stalled complexes resumed movement, and some complexes could even be stalled multiple times this way (Fig. 2C). After recovery from stall, the velocity of bead movement in the low-force region of the trap was similar to that of untreated beads.
before stall. The remaining fraction of RNA polymerase molecules stalled irreversibly in that they did not resume elongation when \( F_{\text{trap}} \) was reduced. Irreversible stalling may be attributable to one or more of several possible causes: The polymerase may have been directly inactivated by the mechanical load, have suffered photodamage from the laser light, or have spontaneously converted into an inactive species (“transcriptional arrest”) similar to that formed during transcriptional stalling induced by NTP depletion (18, 19).

The simplest physical interpretation of reversible stalling is that it corresponds to the situation in which \( F_{\text{trap}} \) has increased to a level where it balances the maximal force that the polymerase can exert (\( F_{\text{stall}} \)), and no further progress is made. When \( F_{\text{trap}} \) is reduced, enzyme activity resumes (20). During both reversible and irreversible stalls, movement sometimes slowed gradually during the approach to \( F_{\text{stall}} \) (for example, the third stall in Fig. 2C), whereas in other cases movement continued at nearly constant velocity, slowing abruptly close to \( F_{\text{stall}} \) (for example, the first and second stalls in Fig. 2C). Transcription complexes display differing biochemical properties depending on the nucleotide sequence of the DNA to which they are bound (21). Abrupt reversible stalling may correspond to the arrival of the enzyme at a template position for which \( F_{\text{stall}} \) is somewhat lower than that of the preceding positions, producing rapid arrest. This interpretation also could explain why \( F_{\text{stall}} \) values determined for multiple stalls of a single complex often differed by more than the experimental uncertainty in measurement (22). It is tempting to speculate that some sites of abrupt stalling might correspond to DNA sequences that trigger cycles of discontinuous elongation (2, 3) or “jumping.” At such sequences, a portion of the RNA polymerase molecule may, during a single nucleotide addition cycle, move along the DNA by \( \sim 10 \) bp, an axial distance of \( \sim 3.4 \) nm (23). These large movements of the enzyme would be expected, all else being equal, to give stall forces significantly lower than those of single base pair movements, because the free energy available from nucleotide addition would be applied over a larger distance. Nucleotide sequence effects on behavior under mechanical load could be studied in future experiments through the use of DNA templates containing homopolymer tracts or direct repeats.

The distributions of \( F_{\text{stall}} \) values were obtained at 1 mM NTPs with 1 \( \mu \text{M} \) PPi. The distributions of reversible and irreversible stall forces were statistically indistinguishable, with values of \( 13.0 \pm 4.0 \) pN (mean \( \pm \) SD, \( n = 8 \)) and \( 12.6 \pm 3.5 \) pN (\( n = 14 \)), respectively. A possible explanation for the similarity of the distributions is that the reversibly stalled state is a precursor of the irreversibly stalled state. In this view, most or all of the stalled complexes are initially stalled reversibly, but a fraction of these are subsequently inactivated and therefore fail to resume elongation when \( F_{\text{trap}} \) is reduced. \( F_{\text{stall}} \) was also determined at two higher concentrations of PPi, (0.5 and 1 mM) with 1 mM NTPs. Increasing the PPi concentration (1 mM slowed transcriptional elongation by about twofold (24), but \( F_{\text{trap}} \) remained nearly constant at 0.5 mM PPi, \( 13.0 \pm 4.1 \) pN (\( n = 5 \)) for reversible and \( 13.1 \pm 2.6 \) pN (\( n = 10 \)) for irreversible stalls; at 1.0 mM PPi, \( 11.5 \pm 2.9 \) pN (\( n = 11 \)) for reversible and \( 9.9 \pm 3.4 \) pN (\( n = 18 \)) for irreversible stalls; this observation may place important constraints on the RNA polymerase force-generation mechanism. In light of this result, stalls observed at all PPi concentrations were pooled (Fig. 3A) to generate a global distribution with mean \( F_{\text{stall}} = 12.3 \pm 3.5 \) pN for all reversible stalls. We previously found that single immobilized transcription complexes exhibit a range of velocities (7). Such heterogeneity among complexes may also contribute to the width of \( F_{\text{stall}} \) distributions. The polymerase is fixed to the glass at random and presumably is not free to rotate. Different spatial orientations of the polymerase with respect to the direction of the applied force might also cause \( F_{\text{stall}} \) to vary from molecule to molecule. A small fraction of the complexes did not stall before reaching the limit of the usable range of the trap (Fig. 3B). The assumption that these complexes would stall at a force larger than the maximum measurable force at the laser power used yields a lower limit estimate of 13.6 pN for the mean reversible \( F_{\text{stall}} \) (25). Despite systematic instrumentation errors for these measurements estimated at \( \sim 30\% \) (22), the \( F_{\text{stall}} \) values for single RNA polymerase molecules are clearly much larger than are forces previously measured for single molecules of other mechanoenzymes: up to 6 pN for kinesin (26) and 3 to 5 pN for myosin (27).

The efficiency of chemomechanical energy conversion in RNA polymerase may be defined as the fraction of the total free energy change of the chemical RNA polymerization reaction (\( \Delta G'_{\text{poly}}} \)) that the enzyme expends to perform mechanical work against an external load. Thermodynamically reversible motors are most efficient as they approach the point of stalling. The free energy available from the chemical reaction varies with the PPi-to-NTP concentration ratio because these are the product and
reactant conditions. For the conditions used, \( \Delta G_{\text{polyn}} \) ranged from -7.2 kcal mol\(^{-1}\) (1 \( \mu M \) PP) to -3.1 kcal mol\(^{-1}\) (1 \( \mu M \) PP) (1). Under the assumption that each NTP consumed advances the polymerase 1 bp on average along the DNA (0.34 nm along the DNA axis), the mean \( F_{\text{stall}} \) estimate corresponds to maximal energy conversion efficiencies of 9, 19, and 22% at 0.001, 0.5, and 1 mM PP, respectively. These efficiencies are comparable to values obtained for biological motors such as kinesin [40 to 60% (11, 28)] or myosin [12 to 42% (27)] (29).

Nucleic acid polymerases carry out biosynthetic reactions and are not ordinarily classified as mechanoenzymes. Nevertheless, our results indicate that individual molecules of E. coli RNA polymerase exert forces and operate under energy conversion efficiencies that are similar to those of prototypical mechanoenzymes, whose specialized function is to generate biologically useful force and motion. Inside living cells, interactions between transcription complexes and cellular structures or DNA-bound proteins create substantial forces that oppose the translocation of polymerases relative to DNA (4, 5). For example, to function in opposition to loads imposed by transcription-induced supercoiling of plasmid DNA in vivo, E. coli RNA polymerase must generate forces estimated at \(-6\) pN (30). Forces of this magnitude are sufficient, for example, to stall solitary molecules of kinesin or myosin, but are nonetheless smaller than the forces achieved by RNA polymerase molecules in vitro. We anticipate that further development of optical measurements on single transcription complexes will allow detailed characterization of the multiple mechanical processes (2, 3, 23) by which RNA polymerase moves along DNA.

REFERENCES AND NOTES

Minimization of a Polypeptide Hormone

Bing Li, Jeff Y. K. Tom, David Oare, Randy Yen, Wayne J. Fairbrother, James A. Wells,* Brian C. Cunningham*

A stepwise approach for reducing the size of a polypeptide hormone, atrial natriuretic peptide (ANP), from 28 residues to 15 while retaining high biopotency is described. Systematic structural and functional analysis identified a discontinuous functional epitope for receptor binding and activation, most of which was placed onto a smaller ring (Cys6 to Cys17) that was created by repositioning the ANP native disulfide bond (Cys7 to Cys23). High affinity was subsequently restored by optimizing the remaining noncritical residues by means of phage display. Residues that flanked the mini-ring structure were then deleted in stages, and affinity losses were rectified by additional phage-sorting experiments. Thus, structural and functional data on hormones, coupled with phage display methods, can be used to shrink the hormones to moieties more amenable to small-molecule design.

The generation of leads for drug design is usually achieved through a laborious discovery process wherein a large number of small molecules are screened for binding to a particular receptor or for modulation of a particular biological response. Although structure-based approaches have been used in some cases for generating candidate molecules directly (1), this approach has been limited to binding sites for small substrate molecules or short, continuous peptide segments.

Protein-protein interactions are crucial events in most biological processes and are therefore important targets for drug design. Such interfaces are generally large (600 to more than 1300 Å²), with 10 to 30 contact side chains on each side of the interface (2). Moreover, each patch of contact residues is presented from peptide segments that are often distant in primary sequence. Mimicking such large and discontinuous binding surfaces with rationally designed small molecules is a daunting prospect, but it may be simplified because only a small subset of contact side chains appears to be necessary for tight binding at these interfaces (3). Displaying these functional epitopes on minimal structured scaffolds may permit smaller candidate compounds to be generated that bind at protein-protein interfaces.

Atrial natriuretic peptide is a 28-residue peptide hormone that is important for regulation of blood pressure and salt balance (4). Smaller ANP peptides produced by the screening of synthetic analogs are at least 500 times weaker in receptor-binding affinity (5). It is likely that the binding of ANP to its signaling receptor is highly sensitive to the conformation of ANP, as indicated by the loss of binding caused by the reduction of its single disulfide bond. Using a constrained scaffold designed to preserve the structural presentation of the critical binding determinants, we present a systematic strategy for reducing the size of ANP while maintaining high binding affinity and biopotency.

The first step in our minimization process (Fig. 1) was to determine which ANP residues are important for binding to the extracellular domain of the natriuretic peptide receptor-A (NPR-A) by alanine-scanning mutagenesis (6). Each of the 28 residues in ANP was converted to alanine, except those comprising the Cys7 to Cys23 disulfide and Ala17. An enzyme-linked immunosorbent assay (ELISA) of these ANP mutants, produced as peptide fusions with gene III coat protein on phage (7), allowed us to rapidly assess the relative binding affinity of the mutants by eliminating the need to purify each peptide to homogeneity. Mutations at only seven positions (Phe6, Met12, Asp13, Arg14, Ile15, Leu16, and Arg23) each resulted in an affinity that was more than 10 times lower than that of wild-type ANP (Fig. 2). These results are consistent with effects on an aortic ring contraction assay reported for substitutions with D-amino acids or alanines in ANP (8).

Five of the seven most important residues (Phe6, Met12, Asp13, Arg14, and Ile15) formed a small functional epitope on one side of the hormone (Fig. 3A). On the basis of the structure of ANP (9), three alternative disulfide forms of the molecule (Cys6 to Cys17, Cys6 to Cys17, and Cys5 to Cys17) were designed to isolate this functional epitope on a smaller disulfide ring and eliminate the native Cys7 to Cys23 disulfide (Fig. 3B). Of these three variants, Cys6 to Cys17 was the best, albeit with an affinity that was more than 100 times lower than...