Picocalorimetry of Transcription by RNA Polymerase

Elio A. Abbondanzieri,* Joshua W. Shaevitz,† and Steven M. Block*‡
*Department of Applied Physics, †Department of Physics, and ‡Department of Biological Sciences, Stanford University, Stanford, California 94305

ABSTRACT Thermal variations can exert dramatic effects on the rates of enzymes. The influence of temperature on RNA polymerase is of particular interest because its transcriptional activity governs general levels of gene expression, and may therefore exhibit pleiotropic effects in cells. Using a custom-modified optical trapping apparatus, we used a tightly focused infrared laser to heat single molecules of *Escherichia coli* RNA polymerase while monitoring transcriptional activity. We found a significant change in rates of transcript elongation with temperature, consistent with a large enthalpic barrier to the condensation reaction associated with RNA polymerization (~13 kcal/mol). In contrast, we found little change in either the frequency or the lifetime of off-pathway, paused states, indicating that the energetic barrier to transcriptional pausing is predominantly entropic.

Received for publication 8 September 2005 and in final form 20 September 2005.

Joshua W. Shaevitz's present address is Dept. of Integrative Biology, University of California, Berkeley, CA 94720.

Address reprint requests and inquiries to Steven M. Block, E-mail: sblock@stanford.edu.

Bacteria such as *Escherichia coli* are capable of growing in a variety of chemical media and over a wide range of ambient temperatures. At fixed temperature, the increase in growth induced by a shift to a richer medium is accompanied by changes in the concentrations of nucleic acids and proteins (1), including an increase in the cellular concentration of RNA polymerase (RNAP). However, if the composition of the growth medium is maintained as the temperature is raised, the resultant increase in growth rate does not lead to detectable changes in the macromolecular contents of the cell (2), provided that temperature remains within the normal range (21–37°C). Because the increase is not accompanied by relative changes in protein or RNA levels, the rates of expression of these components must increase proportionally.

The synthesis of RNA by RNAP provides a universal checkpoint for gene expression in prokaryotic cells. Transcription can be regulated both by controlling initiation at promoter sites or by inducing termination in elongating complexes. In the latter case, transcriptional pausing has been shown to play a central role in regulating termination by coordinating the activity of RNAP with various transcriptional cofactors and enzymes. Pausing was first observed in gel-based measurements of RNAP (3), and more recently in single-molecule measurements (4,5). Temperature could act, in principle, by directly altering rates of elongation or changing the frequency of pausing, or both.

To explore the response of RNAP to temperature, we characterized transcription at the single-molecule level while controlling the thermal environment in the immediate vicinity of the enzyme. Single-molecule approaches are best suited for such a study because they can determine elongation and pausing rates independently, conferring an advantage over biochemical approaches, where active elongation rates are difficult to extract in the presence of pausing (4). We constructed a dual-beam optical trap based on a 1064-nm laser, details of which have been described (6). Control of the thermal microenvironment in the region of the two traps was achieved by adjusting the temperature of the experimental room and thereafter modulating the intensity of the near-infrared trapping light, which is weakly absorbed by water in the buffer and converted into heat. Temperature control based on absorption from a laser beam has been described previously (7–9) and is advantageous because it eliminates the need for heating elements attached to the apparatus, which can add noise. Mao and co-workers (8) reported that laser heating can introduce unwanted convection currents under some circumstances, but this effect is negligible in our system, given the thin depth of the sample chambers employed (~50 μm). The use of localized heating produced by a diffraction-limited laser focus over regions of volume of order 10^{-12} L permits picocalorimetric measurements on individual proteins.

The degree of heating produced by the laser can be inferred sensitively by measuring the change in viscosity of the aqueous buffer, which is strongly temperature-dependent. The viscosity in the immediate vicinity of the trapping centers was determined by measuring the Brownian motion of an optically trapped bead (8,9). Positional power spectra were recorded for a bead held in a weak trap as the intensity of a nearby, empty trap was modulated (Fig. 1 *A*). These spectra were fit to the Lorentzian form:

$$P(f) = \frac{k_B T}{6\pi^3 \eta(T) r \left(\left(\frac{\alpha}{12\pi^2 \eta(T) r} \right)^2 + f^2 \right)},$$

doi: 10.1529/biophysj.105.074195

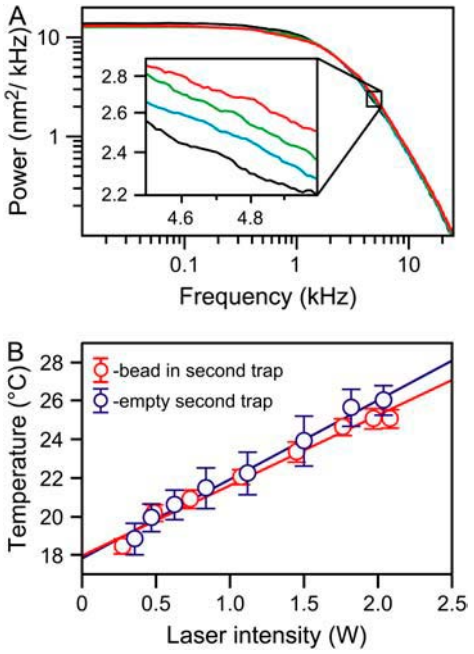

FIGURE 1 **(*A*) Power spectra for a bead held in an optical trap measured while the intensity of a second, nearby trap was modulated (laser intensities, 2.0 W, *red*; 1.4 W, *green*; 0.7 W, *blue*; 0.3 W, *black*). (*B*) Temperatures determined from fits to power spectra (mean ± SE), plotted as a function of laser intensity.**

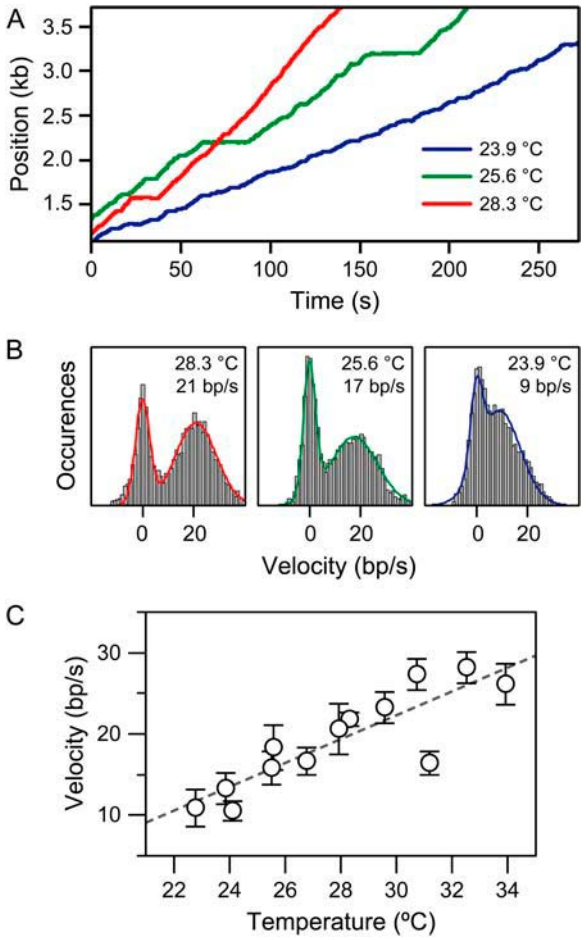

FIGURE 2 **(*A*) Representative records of transcription by RNAP molecules at various temperatures versus time. (*B*) Histograms of velocity derived from the traces in panel *A*, shown with fits to a sum of two Gaussians. (*C*) Plot of RNAP velocity between pauses (mean ± SE) as a function of temperature, from (*N* = 113) records.**

where α is the trap stiffness, r is the bead radius, and $\eta(T)$ is the viscosity, whose temperature dependence is well documented (10). Power spectral measurements were repeated with a 700-nm diameter bead held in the second trap. In both cases, temperature increased linearly with the power directed into the objective (Fig. 1 *B*). The magnitude of heating with no bead in the second trap (4.1 ± 0.5 K/W) was indistinguishable from the heating observed when the second trap held a 700-nm bead (3.6 ± 0.2 K/W). We also found that heating was fairly uniform in the region of the traps, out to a distance of several micrometers (data not shown), consistent with earlier reports (7,8).

Using the calibration curve of Fig. 1 *B*, we measured RNAP transcription rates in single-molecule assays at controlled temperatures within the normal range. The transcription assay has been described previously (6). The position of RNAP along a DNA template derived from the rpo*B* gene (4) was digitized at 2 kHz as the enzyme was held under a controlled load of ~8 pN. Three representative records are shown in Fig. 2 *A*. Histograms of velocity were generated from the derivatives of the records: these display two clear peaks, well-fit by Gaussians (Fig. 2 *B*). The first is centered at zero and corresponds to the pause state, while the second is centered at a positive velocity and represents the elongation rate between pauses (4). A plot of 113 individual records taken over a range of temperatures (Fig. 2 *C*) demonstrates that the elongation rate is highly sensitive to temperature, increasing by a factor of more than two over the range studied.

Temperature might also affect the rate constants associated with entry into or exit from off-pathway pause states. To assess this possibility, we ran a pause detection algorithm on individual records, as described previously (4). Pauses were scored whenever the velocity (computed with a second-order Savitsky-Golay filter with a time constant of 2 s) fell below a threshold. This algorithm detects pauses longer than 1 s with >95% confidence. Prior work has shown that long pauses ($t > 20$ s) occur infrequently, via a separate mechanism (6). The infrequency of long pauses made it impractical to gather extensive statistics on these: we therefore restricted our analysis to short pauses, which have a characteristic time constant of ~3 s (4).

A simplified kinetic model for RNAP is presented in Fig. 3 *A*. The rate of forward elongation, k_e, is in kinetic competition with the rate of entry into the paused state, k_p. The lifetime of a pause is determined by the rate of escape, k_{-p}. We estimated k_p from records by dividing the number of

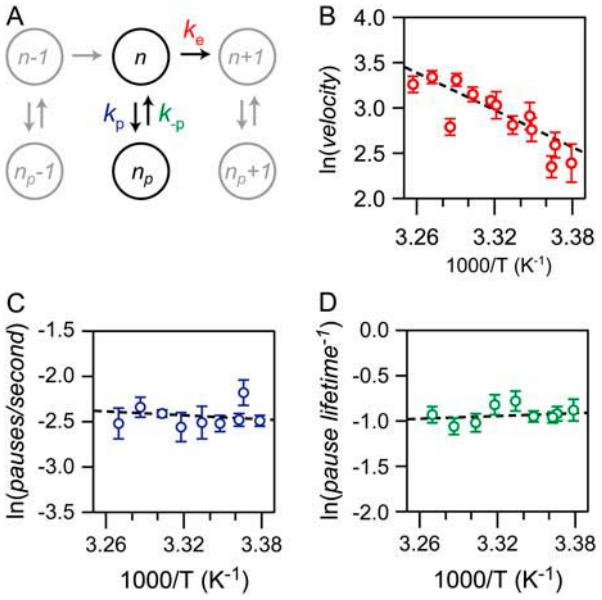

FIGURE 3 (*A*) Simplified kinetic model for pausing; short pauses (*bottom*) branch off the main elongation pathway (*top*); *n* denotes length of the transcript. (*B–D*) van 't Hoff plots of mean velocity between pauses, rate of entry into the paused state, and rate of escape from the paused state, respectively.

pauses detected by the total elongation time between the pauses. The value of k_{-p} was estimated from the inverse mean pause lifetime. In Fig. 3, *B–D*, the logarithm of the rates of transcriptional elongation, pause entry, and pause escape are plotted against inverse temperature. According to transition state theory (11), this relationship is supplied by the van 't Hoff equation: $\ln(k) = -(\Delta H/k_B T - \Delta S/k_B) + \ln(h/k_B T)$, where ΔH and ΔS represent the enthalpic and entropic contributions to the rate-limiting transition associated with transcription, respectively, k_B is Boltzmann's constant, and h is Planck's constant. Fits to this relation revealed that the rate of elongation has a large enthalpic component (91 $\pm$ 11 pN nm; 13 $\pm$ 2 kcal/mol). Interestingly, this value corresponds closely to the enthalpic component measured independently for overall *E. coli* growth (92 pN nm; 13 kcal/mol) (11). In contrast, the enthalpies associated with the rates of pause entry (6 $\pm$ 11 pN nm; 1 $\pm$ 2 kcal/mol) and escape (-11 ± 11 pN nm; -2 ± 2 kcal/mol) are zero within experimental error. These results suggest that short pauses correspond to an entropic transition within the enzyme.

Experimentally, we found that rates of elongation increased with temperature by the same relative amounts as the general growth rates for *E. coli*. This result may explain why no increase in cellular RNAP enzyme levels is required for concomitant increases in the growth rate with temperature, in contrast to the higher RNAP levels associated with increased growth at a fixed temperature in richer media (1,11). As the elongation rate increases, RNAP can cycle more quickly through an operon and reinitiate at a new promoter. Therefore, proteins will be produced more rapidly to keep pace with the increased growth rate. In this fashion, temperature exerts a global regulatory action via RNAP.

ACKNOWLEDGMENTS

The authors thank Robert Landick for the gift of RNAP, and Polly Fordyce and Anne Meyer for comments on the manuscript.

This work was supported by grants to S.M.B. from the National Institute of General Medical Sciences of the National Institutes of Health.

REFERENCES and FOOTNOTES

1. Bremer, H., and P. P. Dennis. 1996. Modulation of the chemical composition and other parameters of the cell growth rate. *In* Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd ed. F. C. Neidhardt, R. Curtiss, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, editors. ASM Press, Washington, DC. 1553–1569.
2. Schaechter, M., O. Maaloe, and N. O. Kjeldgaard. 1958. Dependency on medium and temperature of cell size and chemical composition during balanced grown of Salmonella typhimurium. *J. Gen. Microbiol.* 19: 592–606.
3. Levin, J. R., and M. J. Chamberlin. 1987. Mapping and characterization of transcriptional pause sites in the early genetic region of bacteriophage T7. *J. Mol. Biol.* 196:61–84.
4. Neuman, K. C., E. A. Abbondanzieri, R. Landick, J. Gelles, and S. M. Block. 2003. Ubiquitous transcriptional pausing is independent of RNA polymerase backtracking. *Cell.* 115:437–447.
5. Adelman, K., A. La Porta, T. J. Santangelo, J. T. Lis, J. W. Roberts, and M. D. Wang. 2002. Single molecule analysis of RNA polymerase elongation reveals uniform kinetic behavior. *Proc. Natl. Acad. Sci. USA.* 99: 13538–13543.
6. Shaevitz, J. W., E. A. Abbondanzieri, R. Landick, and S. M. Block. 2003. Backtracking by single RNA polymerase molecules observed at near-base-pair resolution. *Nature.* 426:684–687.
7. Celliers, P. M., and J. Conia. 2000. Measurement of localized heating in the focus of an optical trap. *Appl. Opt.* 39:3396–3407.
8. Mao, H., J. R. Arias-Gonzalez, S. B. Smith, I. Tinoco, Jr., and C. Bustamante. 2005. Temperature control methods in a laser tweezers system. *Biophys. J.* 89:1308–1316.
9. Peterman, E. J., F. Gittes, and C. F. Schmidt. 2003. Laser-induced heating in optical traps. *Biophys. J.* 84:1308–1316.
10. Press, C. R. C. 1977. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL.
11. Ingraham, J. L., and A. G. Marr. 1996. Effect of temperature, pressure, pH, and osmotic stress on growth. *In* Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd ed. F. C. Neidhardt, R. Curtiss, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger, editors. ASM Press, Washington, DC. 1570–1578.