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Cold Sensitivity of Thermophilic and Mesophilic RNA Polymerases

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RNA polymerase from mesophilic *Deinococcus radiodurans* displays the same cold sensitivity of promoter opening as RNA polymerase from the closely related thermophilic *Thermus aquaticus*. This suggests that, contrary to the accepted view, cold sensitivity of promoter opening by thermophilic RNA polymerases may not be a consequence of their thermostability.

Thermophilic enzymes are known to work at low temperatures much slower than their mesophilic counterparts. Such cold sensitivity is believed to be a penalty for their more rigid structure, which ensures high protein thermostability. The increased rigidity of thermophilic enzymes is supposed to reduce their conformational mobility at low temperatures and to block structural changes required for catalysis (5, 16). Accordingly, it is anticipated that comparative study of thermophilic enzymes may help to characterize conformational dynamics involved in catalysis.

Promoter opening by RNA polymerase (RNAP)—a crucial step of transcription involving DNA melting around the point of RNA initiation—seemed to conform to this paradigm. It was demonstrated long ago that RNAPs from moderately thermophilic *Bacillus* species opened promoters at higher temperatures than the RNAP from mesophilic *Escherichia coli* (13, 15); both the core and σ subunit were responsible for the observed cold sensitivity (12). Using chemical probes, it was shown that thermostable RNAP from *Thermotoga maritima* opened promoters through a series of isomerization events that were very similar to those observed with the *E. coli* enzyme but that occurred at higher temperatures (10). Recent studies of *Thermus thermophilus* (18) and *Thermus aquaticus* RNAPs (11) confirmed cold sensitivity of promoter opening by thermophilic RNAPs relative to that by *E. coli* RNAP. It was concluded that cold sensitivity of promoter opening by thermophilic RNAPs was a penalty for adaptation to high temperatures, resulting in more rigid protein structure (18). This conclusion was based, however, on a taciturn assumption that all mesophilic RNAPs were similar to *E. coli* RNAP, i.e., they were cold resistant. However, scrutiny of limited information available about RNAPs from mesophilic bacteria other than *E. coli* hinted that this may not be the case (8, 17). To clarify the issue, we compared RNAPs from *T. aquaticus* and *Deinococcus radiodurans*. *T. aquaticus* RNAP was chosen because X-ray structures of *T. aquaticus* and closely related *T. thermophilus* RNAPs were available. *D. radiodurans* RNAP was chosen because this bacterium is the closest mesophilic counterpart of *T. aquaticus* (7, 9). The strong phylogenetic relationship between *T. aquaticus* and *D. radiodurans* suggested that most differences between their RNAPs were likely to result from different temperature adaptations of these bacteria. Surprisingly, many characteristics ascribed previously to thermophilic RNAPs were also found in the mesophilic *D. radiodurans* enzyme. This demonstrated that comparison of thermophilic RNAPs with *E. coli* RNAP as the only representative of mesophilic enzymes indeed may lead to misleading conclusions.

We isolated *E. coli* and *D. radiodurans* cores from corresponding cells essentially as described previously (1, 3, 4). Recombinant *T. aquaticus* core and *E. coli* and *T. aquaticus* σ subunits were overproduced in *E. coli* cells containing appropriate plasmids (1, 11). The *D. radiodurans rpoD* gene was amplified from the genomic sequence (GenBank accession number NP_294640.1) and cloned into the pET28 plasmid. The recombinant protein was overproduced and purified by analogy with the *T. aquaticus* σ^A subunit. *E. coli*, *T. aquaticus*, and *D. radiodurans* holoenzymes were reconstituted by mixing core enzymes with a fivefold excess of σ subunit. We then compared promoter binding, open complex formation, elongation, and termination by these RNAPs. We found that *T. aquaticus* RNAP differed from *E. coli* RNAP in most assays used. It displayed reduced stability of promoter complexes at the optimal temperature, was more resistant to rifampin and more sensitive to streptolydigin than the *E. coli* enzyme, was less prone to abortive RNA synthesis, and showed specific differences in RNA termination (Table 1). As expected, *D. radiodurans* RNAP was thermosensitive and had a mesophilic temper-

TABLE 1. Properties of *E. coli*, *T. aquaticus*, and *D. radiodurans* RNAPs

Characteristic	RNAP		
	E. coli	*T. aquaticus*	*D. radiodurans*
Temperature optimum[a] (°C)	37	60	37
Residual activity (%) after heating for 10 min at 65°C	1.5	74	2
Ratio of activities (%) at 20 and 45°C	53	0.25	0.6
Open complex half-life time[b]	>30 min	<20 s	<20 s
Sensitivity to rifampin[c] (μg/ml)	0.1	100	100
Sensitivity to streptolydigin[c] (μg/ml)	>10	<0.1	<0.1

[a] Measured in a multiple-round transcription assay on a template containing T7 A1 promoter and λ tR2 terminator (14); the activity was quantified as the sum of runoff and terminated RNA products.
[b] Measured in the transcription assay at the optimum temperature in the presence of 5 μg of heparin/ml.
[c] Concentration of antibiotic required to inhibit 90% of the RNAP activity.

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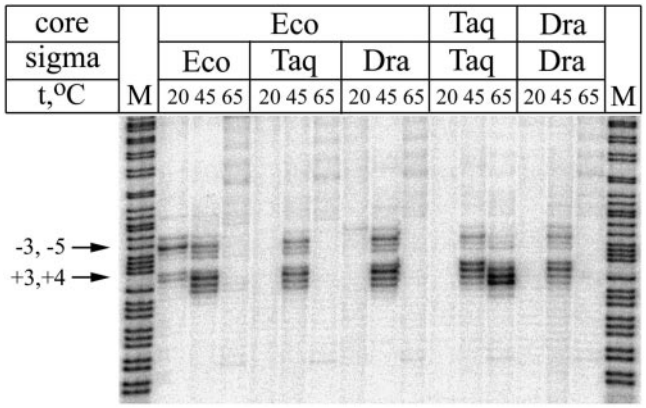

FIG. 1. Permanganate footprints of *E. coli* (Eco), *D. radiodurans* (Dra), and *T. aquaticus* (Taq) RNAPs and hybrid holoenzymes on the nontemplate strand of *lac*UV5 promoter at 20, 45, and 65°C. Arrows with numbers indicate the positions of hyperreactive thymine residues relative to the starting point of transcription. M lanes are A+G cleavage markers.

ature optimum. At the same time, the *D. radiodurans* enzyme behaved very similarly to *T. aquaticus* RNAP in most other transcription assays (Table 1 and data not shown).

Most importantly, *T. aquaticus* and *D. radiodurans* RNAPs possessed a similar cold sensitivity of promoter opening in $KMnO_4$ probing experiments that detect thymines in single-stranded but not in double-stranded DNA. We studied DNA melting by *E. coli*, *T. aquaticus*, and *D. radiodurans* RNAPs in *lac*UV5 promoter complexes at different temperatures using end-labeled DNA fragment as described elsewhere (2). In brief, holoenzyme of RNAP (100 nM core plus 500 nM σ) was incubated with the promoter DNA (10 nM) in transcription buffer (40 mM Tris-HCl [pH 7.9], 40 mM KCl, 10 mM $MgCl_2$) for 10 min at the temperatures indicated and treated for 10 s with 5 mM $KMnO_4$. The modified thymines were detected by piperidine strand cleavage (2). All three RNAPs melted DNA around the starting point of transcription at 45°C. However, only *E. coli* RNAP opened the promoter at 20°C (Fig. 1). At 65°C, only *T. aquaticus* RNAP melted the promoter, which conformed to its higher thermostability in comparison with the mesophilic RNAPs.

To determine the role of the core and σ subunits in cold sensitivity of promoter opening, we performed $KMnO_4$ footprinting experiments with hybrid core-σ holoenzymes. Unfortunately, the *E. coli* σ^{70} subunit did not form active holoenzymes with *T. aquaticus* or *D. radiodurans* core polymerases (data not shown) (11). However, holoenzymes containing *E. coli* core and *T. aquaticus* or *D. radiodurans* σ^A subunits were active and opened *lac*UV5 promoter at 45°C but not at 20°C (Fig. 1). Thus, cold sensitivity of promoter opening by *T. aquaticus* and *D. radiodurans* RNAPs is apparently determined primarily by their σ subunits. It should be emphasized that the $KMnO_4$ probing experiments presented here assessed promoter opening under equilibrium conditions, while it remains possible that *T. aquaticus* and *D. radiodurans* RNAPs do possess some differences on intermediate stages of the open complex formation.

It was previously shown that thermophilic *T. thermophilus* and *T. aquaticus* RNAPs possess certain defects in RNA elongation compared with the *E. coli* enzyme (11, 18). To measure the rate of RNA synthesis by *E. coli*, *T. aquaticus*, and *D. radiodurans* RNAPs, we preformed stalled elongation complexes on a DNA template containing T7 A1 promoter and λ tR2 terminator (14) and followed RNA elongation at different temperatures. As is seen from Fig. 2, *T. aquaticus* and *D. radiodurans* enzymes reached the tR2 terminator at 37°C as fast as the *E. coli* enzyme. At 0°C, it took about 15 min to reach the terminator for the *E. coli* and *D. radiodurans* enzymes at 100 μM NTP, while the *T. aquaticus* enzyme failed to reach the terminator even after 24 h at 1 mM NTP. At lower substrate concentrations (100 μM NTP), *T. aquaticus* RNAP elongation

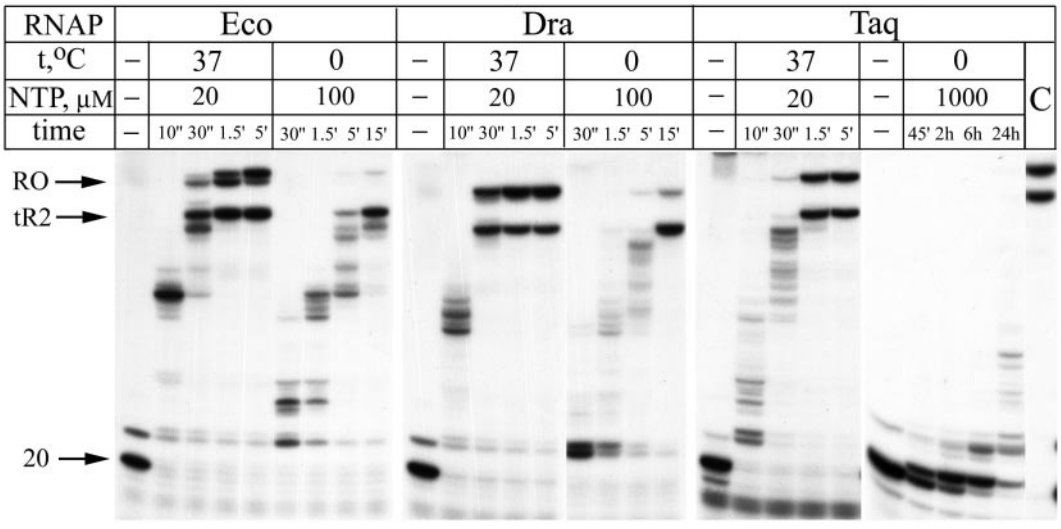

FIG. 2. RNA elongation by *E. coli* (Eco), *D. radiodurans* (Dra), and *T. aquaticus* (Taq) RNAPs on the T7 A1 promoter fragment followed by λ tR2 terminator. The starting 20-mer RNA, runoff, and terminated transcripts are shown by arrows. Elongation was measured at different NTP concentrations at 37 and 0°C. The sample in lane C was incubated for 24 h at 0°C with 1 mM NTP and then transferred to 37°C for the additional 5 min.

was immeasurably slow (data not shown). Control experiments showed that the *T. aquaticus* RNAP elongation complex was not dissociated or irreversibly inactivated at 0°C and was fully active after transfer to 37°C (Fig. 2, lane C). The dramatic defect in *T. aquaticus* RNAP elongation observed at 0°C suggests that elongation requires conformational changes that are severely impeded at low temperature.

Our data show that the dramatic cold sensitivity of elongation displayed by thermophilic *T. aquaticus* RNAP may be related to thermal adaptation. Since *D. radiodurans* RNAP had the same elongation rates as the *E. coli* enzyme at all temperatures tested, one can conclude that the cold sensitivity of RNA elongation is the only *T. aquaticus* RNAP "defect" which is correlated with its thermophily and thus could be regarded as the penalty for its thermostability. In contrast, other functional differences between *T. aquaticus* and *E. coli* RNAPs, including cold sensitivity of promoter opening by the thermophilic enzyme, may not be consequences of adaptation to high temperature. These differences may be a neutral character or reflect adaptation to some unknown condition common to *T. aquaticus* and *D. radiodurans* and some other bacteria. The high level of homology between *T. aquaticus* and *D. radiodurans* RNAPs implies that that the nature of the observed promoter opening defects is similar for both enzymes. At the same time, we also cannot exclude the possibility that these features arise from different sequence and structure determinants that have evolved independently in these bacteria. Ignorance on the adaptive role of the functional peculiarities of *T. aquaticus* and *D. radiodurans* RNAPs does not prevent use of these enzymes as natural mutants that have diverged from *E. coli* by evolutionary design. As was shown for a few other enzymes (6), such mutants may provide useful phenotypes for structural studies that cannot be obtained with standard genetic approaches.

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