METHODS

Structure determination and refinement. The *T. thermophilus* RNAP holoenzyme was purified and crystallized as described previously[20]. To obtain the complex crystals, the crystals of the apo-holoenzyme were transferred for 3 h into the drops containing collection buffer and 2 mM of dMyx[4]. The data were collected at beam line GM/CA-CAT (Advanced Photon Source, Argonne, USA). The crystals, although belonging to the same space group $P3_2$, had distinct unit cell parameters, $a = b = 235$ Å, $c = 255$ Å, as compared to the two previously studied *T. thermophilus* RNAP crystal forms; form I ($a = b = 236$ Å, $c = 250$ Å)[20–22] and form II ($a = b = 240$ Å, $c = 253$ Å)[23–25]. The data were processed using the HKL2000 data processing package (Supplementary Table 1)[26]. As all previous holo-RNAP crystals, the crystals of the RNAP–dMyx complex were characterized by the perfect merohedral twinning coupled with the non-crystallographic symmetry[20]. Refinement, therefore, was carried out using the twinning option of the CNS program (Supplementary Table 1)[27]. The RNAP structure from the RNAP–Tgt complex (PDB accession 2BE5), from which the bridge helix (β′1067–1104) and the switch-2 segment (β′602–621) were omitted, was used as a starting model. Zonal scaling correction[10] substantially improved the initial R_{free} and the quality of the electron density. The $|2F_{obs} - F_{calc}|$ electron density map calculated after the rigid body and B-factor refinement steps revealed the undistorted bridge helix (characteristic of the crystal form II, see earlier), straightening of the switch-2 α-helix and a very clear electron density for dMyx. However, the unfolded C-terminal portion of the switch-2 (residues β′613–619) structure was represented by a fragmentary electron density that allowed for alternative interpretations. After several trials, using the reduced level of the electron density (~0.8σ, which was still above the noise) we were able to build a proper model that was characterized by a good, continuous electron density in the $|2F_{obs} - F_{calc}|$ electron density map for both the side- and main-chain atoms (note that all the previous partially wrong models did not improve the quality of the electron density in this region). After modelling of dMyx and switch-2 structures in the omit electron density, several rounds of the B-factor, positional, simulated annealing refinements and water 'pick' and water 'delete' procedures, alternating by manual model building using the program O[28] yielded a final R_{factor} of 24.0% and R_{free} of 27.0% for the RNAP–dMyx complex at 2.7 Å resolution (Supplementary Table 1). The final model was of high quality as shown by the simulated annealing omit electron density map calculated for dMyx and the switch-2 motif (Supplementary Fig. 1). Structural figures were prepared using programs Molscript[29], Bobscript[30] and Raster3D[31].

Isolation and assay of mutant *E. coli* RNAPs. Core wild-type and mutationally altered RNAPs were purified as described previously[18] except that the ionic strength was maintained at or above 0.2 M at all chromatographic steps. Overexpression plasmids for β' R339A (pIA830), β E1279A (pIA870), β S1322E (pIA878), β' G336A (pIA880), β' F338A (pIA881), β' K345A (pIA882), β' Δ333-335G (pIA883), β' K334A (pIA879) and β' R337A (pGB055) were constructed by site-directed mutagenesis and the sequenced fragments were recloned into pVS10-based vectors[18]. Holoenzymes were reconstituted with the twofold molar excess of σ^{70}. For steady-state abortive initiation assays, holo RNAP (20 nM) in 16 μl of 20 mM Tris-acetate, 20 mM sodium acetate, 2 mM magnesium acetate, 5% glycerol, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, pH 7.9, 1 μM σ^{70}, were supplemented with desired concentration of dMyx (2 μl) and incubated for 15 min at 37 °C. Transcription was initiated by adding linear T7A1 promoter template (100 nM), ApU (200 μM), CTP (25 μM) and 3 μCi [α^{32}P]-CTP (final reaction volume 20 μl). Reactions were allowed to proceed for 15 min at 37 °C and quenched by the addition of an equal volume of saturated urea in 90 mM Tris-borate, pH 8.3, 20 mM EDTA. Products were analysed on 7 M urea, 12% (w/v) acrylamide:bisacrylamide (19:1) denaturing gels and RNA quantities were determined from Phosphorimager scans of the gels. dMyx IC_{50} values for wild-type and variant RNAPs were determined by fitting concentration dependencies to hyperbolic function. The assay was repeated at least three times for each variant tested.

Footprinting analysis. A linear 153-bp DNA fragment containing λP_R promoter was made by PCR amplification using pIA226 (ref. 25) as a template with primers 17 (5′-CGTTAAATCTATCACCGCAAGGG) and 138 (5′-ATCGCCTGAAAGACTAGTCAGG). The top (non-template) DNA strand primer (number 17) was end-labelled with [^{32}P]-γATP (Perkin Elmer) and polynucleotide kinase (Epicentre) and purified using G-50 spin columns (GE Healthcare). PCR products were gel-purified using a kit (Promega). Sequencing reactions were performed using the same labelled primer with a SequiTherm kit (Epicentre). For DNaseI protection experiments, wild-type holo *E. coli* RNAP (400 nM) was pre-incubated with 1 μM of Myx or an equal volume of 50% ethanol for 15 min at 37 °C in GBB buffer (20 mM Tris-HCl, 14 mM $MgCl_2$, 20 mM NaCl, 5% glycerol, 1 mM DTT, 0.1 mM EDTA, pH 7.9) supplemented with 1 mM $CaCl_2$. A labelled λP_R promoter fragment was added (at 20 nM) and the reaction was incubated for a further 10 min. Samples were shifted to room temperature (22 °C) and treated with 0.002 U of DNaseI (Roche, 10 U μl^{-1}) for 1 min. The reaction was stopped by the addition of an equal volume of buffer containing 15 mM EDTA and 8 M urea. For potassium permanganate probing, holo RNAP (400 nM) was pre-incubated with 1 μM of myxopyronin or an equal volume of 50% ethanol, 0.5% dimethylsulphoxide for 15 min at 37 °C in GBB buffer without reducing agents. Labelled λP_R promoter fragment was added (at 20 nM), and the reaction was incubated for a further 10 min. Samples were shifted to room temperature and treated with $KMnO_4$ at a final concentration of 10 mM for 60 s. The reaction was stopped by the addition of 5× stop buffer (1.5 M sodium acetate, pH 5.2, 80 mM EDTA, 6 M β-mercaptoethanol), samples were subjected to phenol-chloroform extraction and precipitated with ethanol. The pellet was dissolved in 20 μl of water and incubated with 100 μl of 0.5 M piperidine at 95 °C for 20 min. After another round of ethanol precipitation, DNA was dissolved on 96% formamide. Samples were heated at 95 °C for 3 min and analysed on 7 M urea, 8% (w/v) acrylamide:bisacrylamide (19:1) denaturing gels.

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