Minireview

A bridge to transcription by RNA polymerase
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Abstract

A comprehensive survey of single amino-acid substitution mutations critical for RNA polymerase function published in *Journal of Biology* supports a proposed mechanism for polymerase action in which movement of the polymerase 'bridge helix' promotes transcriptional activity in cooperation with a critical substrate-interaction domain, the 'trigger loop'.

X-ray crystallographic studies on multisubunit RNA polymerases (RNAPs) from eukaryotes, bacteria and archaea have revealed highly related enzymes with structurally conserved active sites. For eukaryotic and bacterial enzymes, crystal structures of transcribing complexes have been solved, showing the locations of the DNA template, the nascent RNA product and the substrate-binding site. Structural studies have their limitations, however, which makes a comprehensive functional screen of amino-acid substitutions in domains critical for polymerase action published in *Journal of Biology* especially useful. The results of the study by Robert Weinzierl's group (Tan *et al.* [1]) are in keeping with previous structural work and will be a valuable resource for interpreting future structural, single molecule and molecular modeling experiments.

Structural information as the foundation for functional studies
Initial structural studies on the prokaryotic and eukaryotic enzymes identified a conserved alpha-helical domain, termed the bridge helix, that spans between two main lobes of the enzyme (for a useful bibliography of the structural literature

on RNA polymerase, see Tan *et al.* [1]). Further studies revealed interactions of this helix with the DNA template, distorting its path adjacent to the nucleotide-addition site. Moreover, the bridge helix appeared in two separate conformations - 'straight' in a eukaryotic RNAP structure and 'kinked' in a bacterial RNAP structure - raising the possibility that the conformational dynamics of the bridge might directly control enzyme translocation: that is, bending of the bridge helix might accompany movement of the polymerase along the DNA template.

Lately, attention has turned from the bridge helix to an adjacent domain of the enzyme, the trigger loop (Figure 1). This loop is mobile or unstructured in many RNAP crystal structures and appears conformationally flexible. In recent structures of transcribing complexes, the trigger loop has been seen to interact directly with template-specified nucleotide substrates [2,3], suggesting critical roles in catalysis and substrate selection. Indeed, mutations in trigger-loop residues alter elongation rate, transcriptional pausing, response to regulators, substrate selection and transcriptional fidelity [4]. The loop makes extensive contacts with the bridge helix, also raising the possibility that the concerted actions of these two
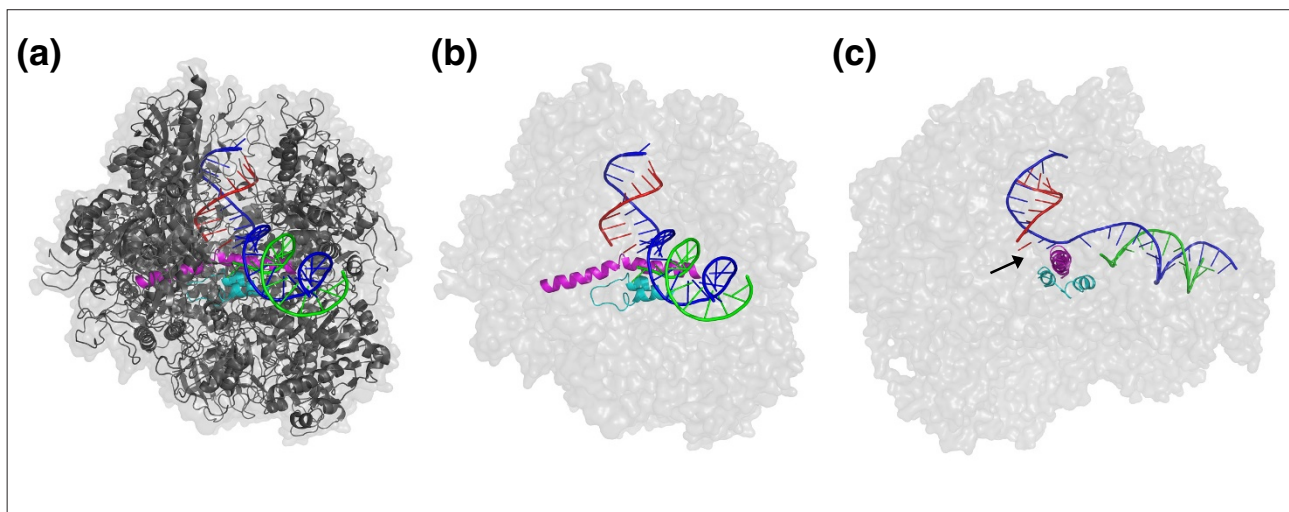
Figure 1
RNA polymerase II elongation complex showing positions of bridge helix (BH) and trigger loop (TL). **(a)** Cartoon representation of 10-subunit Pol II elongation complex (PDB:2e2h) [3] with protein shown in charcoal gray, template DNA in blue, RNA product in red, and non-template DNA in green. Buried within, near the active site, are the TL (cyan) and BH (magenta). **(b)** Transparent view of (a) showing the position of the BH and TL relative to the nucleic acid scaffold within Pol II. **(c)** Rotated view of (b) showing the position of the BH and TL relative to the 3' end of the RNA product (the site of transcript elongation, shown by an arrow). This figure was created with PyMOL.

domains may underlie key RNAP dynamics during the nucleotide-addition cycle.

Structural studies have important limitations in regard to understanding the transcription mechanism at the molecular level. First, transcribing complexes designed for crystallographic studies are unavoidably compromised by alterations made to prevent phosphodiester bond formation. In some structural studies of eukaryotic RNAPs, as with those of numerous DNA polymerases, putative reaction intermediates contain 3'-deoxynucleotide-terminated nucleic acid primers to prevent chain elongation [3,5]. In other eukaryotic RNAP structures [6], and in a structure of a bacterial RNAP transcribing complex [2], non-hydrolyzable substrates were used to prevent phosphodiester bond formation. In either case, such crippled elongation complexes may differ from native complexes in critical aspects.

A second limitation of crystal structures is that they only provide snapshots of stable conformations, failing to fully reveal the dynamics involved in catalysis of phosphodiester bond formation and translocation of RNAP along the template. Current structures of transcribing RNAPs have not attained atomic resolution, placing the reaction mechanism out of reach. Indeed, as modeled, eukaryotic RNAP [3,5,6] and bacterial elongation complexes [2] containing bound substrates differ in putatively critical interactions between trigger loop and nucleotide that could have profound consequences for the RNAP catalytic mechanism. In light of these shortcomings, biochemical studies such as those of Tan *et al.* [1] are especially helpful.

An automated platform for functional studies: the RNA polymerase factory

The current study by Tan *et al.* uses a highly automated, high-throughput approach for the production and characterization of RNAP variants developed previously by Weinzierl and colleagues [7]. Their approach takes advantage of a completely recombinant system for production of the RNAP from the archaeon *Methanocaldococcus jannaschii*. This recombinant archaeal polymerase is composed of nine subunits, A', A", B', B", D, H, L, N and P (the other three subunits of the native enzyme showed no detectable effect on function *in vitro* and were therefore omitted). Recombinant RNAPs may be expressed, purified, assembled and characterized in batches of 96 RNAP variants over 1-2 days, making large-scale analysis of systematically designed RNAP substitution mutants feasible. In addition, the approach is highly automated and can probably be implemented for the study of any number of enzymatic systems, thus making it a generally useful technology for protein-engineering studies.

In the 'RNA polymerase factory', all amino-acid substitutions at a number of positions can be evaluated in parallel, giving an essentially unbiased approach to structure-function

analysis that is not necessarily based on pre-existing structural information or limited by numbers of mutants that can be evaluated. Such high-throughput evaluation does not have to rely on *in vivo* genetic identification of variants, which can be limited by methods of mutagenesis that are able to generate only subsets of all possible substitutions, or by traditional site-directed mutagenesis approaches that rely on prior rationales for the design of variants.

Gain-of-function mutants: the bridge and trigger may work together

In previous proof-of-principle work [7], Weinzierl and colleagues applied their automated analysis to all 19 amino-acid substitution variants for *M. jannaschii* RNAP subunit A' (*mj*A') residue G825, within the bridge helix domain. In their current study [1], they extend this analysis to an astonishing 323 variants within the bridge helix, comprising all 19 single amino-acid substitutions within a 17 amino-acid stretch of the helix. The authors also characterize an additional 38 substitutions, encompassing all 19 single amino-acid substitutions of trigger-loop residues *mj*A" G72 and *mj*A" I98.

In regard to elucidating the interactions between bridge helix and trigger loop, it is fascinating that the authors identify a large number of super-activating substitutions within the bridge helix as well as some within the trigger loop. It was already known that certain trigger-loop substitutions in bacterial RNAP and yeast RNA polymerase II (Pol II) allow the enzymes to transcribe more quickly than wild-type variants. In the study by Tan *et al.* [1] gain-of-function substitutions are present within the region observed to bend in crystal structures and along one side of the helix facing the trigger loop. Double-substitution mutants containing gain-of-function substitutions in both bridge helix and trigger loop have no greater gain of activity than the most severe single substitution, suggesting that the two domains function together to promote transcription.

Functional evidence for bridge helix dynamics in the nucleotide-addition cycle

As mentioned above, the bridge helix can be observed in a number of conformations in crystal structures, with a kink or a bend in various positions (Figure 2). In structures of *Thermus thermophilus* (*Tth*) RNAP in the absence of nucleic acids, the kink is quite pronounced [4,8]. Movement of the trigger loop towards the bridge helix in a *Saccharomyces cerevisiae* RNAP also appears to perturb the helix by altering its path in the direction of the observed kink, although only to a small extent [3]. In each case, the bend is in the same direction - away from the trigger loop. A kinked bridge helix has not yet been observed in a structure of the *Tth* RNAP
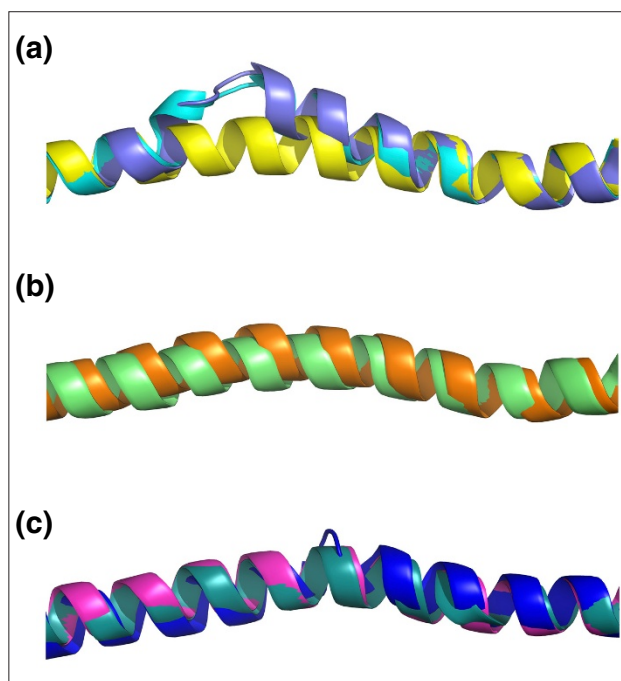
Figure 2
Conformations of the bridge helix (BH) observed in crystal structures of multisubunit RNAPs. **(a)** Kinked orientation of the BH in some *Tth* RNAP crystal structures. BH from *Tth* RNAP holoenzyme without nucleic acids [PDB:1iw7] (cyan) [8]. BH from *Tth* holoenzyme without nucleic acids [PDB:2cw0] (slate blue) [9]. BH from *Tth* holoenzyme without nucleic acids in the presence of streptolydigin [PDB:1zyr] (yellow) [9]. **(b)** Straight orientation of the BH in *Tth* elongation complex structures with or without nucleoside triphosphate (NTP) substrate. BH from the *Tth* transcribing complex without NTP substrate (the trigger loop (TL) in this structure is in the 'out' position away from the substrate-addition site) [PDB:2o5i] (orange) [10]. BH from *Tth* elongation complex with NTP substrate (the TL is folded 'in' and contacting the NTP substrate in this structure) [PDB:2o5j] (lime green) [2]. **(c)** Mild perturbation of the Pol II BH in elongation complex with matched NTP substrate in position for addition. BH from *S. cerevisiae* (*Sce*) Pol II elongation complex without substrate, [PDB:1i6h] (magenta) [11]. BH from the *Sce* Pol II elongation complex with mismatched substrate (the TL is in the 'out' position away from substrate-addition site in this structure) [PDB:1r9t] (teal) [5]. BH from the *Sce* Pol II elongation complex with matched substrate (the TL is folded 'in' and contacting the NTP substrate in this structure) [PDB:2e2h] (blue) [3]. This figure was created with PyMOL.

elongation complex, raising the possibility that an extreme bridge helix kink may not necessarily function in transcription elongation. The relative flexibility of the bridge helix, perhaps not unexpected for such an isolated helix, is underscored by the results of Tan *et al.* [1], who show that the bridge helix can be somewhat tolerant to helix-breaking prolyl substitutions along its length, including one substitution at the position of the observed helix kink (*mj*A' S824P) that super-activates RNAP. Furthermore, kinked bridge helix structures reveal an

interaction between two residues of the helix (analogs of *mj*A' residues 823 and 829) that may stabilize the kinked conformation. Substitutions at either of these putatively kink-stabilizing positions compromise RNAP activity to various extents [1]. Notably, re-establishment of a possible interaction between the two substituted positions results in a moderate restoration of activity, consistent with an on-pathway role for the observed interaction. These results suggest that the kinked bridge helix promotes RNAP activity, but whether in catalysis or translocation remains to be determined.

Future dissection of RNA polymerase function

The RNA polymerase factory has proved its usefulness for the characterization of a large number of RNAP variants. The next stage of this approach is likely to include comprehensive analysis of trigger-loop residues and active-site residues predicted to contact nucleotide substrates. A subset of the mutants identified by Tan *et al.* should be characterized with more traditional assays that are able to directly measure RNAP elongation rate, substrate selection and propensity for pausing. Information on such a wide spectrum of mutants will benefit single-molecule studies in which RNAP variants with specific defects can be used to probe models of the transcription mechanism. In addition, molecular modeling studies that aim to accurately describe and elucidate the RNAP mechanism can be tested for recapitulation of phenotypes demonstrated for a wide range of RNAP variants, such as those described by Tan *et al.*. Finally, biophysical studies able to directly probe and characterize RNAP active-site dynamics, such as bridge helix and trigger loop movement, and how such movement is impacted by substrate binding and perturbation of RNAP structure are likely to be important for understanding of the RNAP mechanism at the near-atomic level. An eventual complete elucidation of the RNAP mechanism, specifically, the identification of the entire retinue of RNAP amino acids required for catalysis and translocation, will be a first step for understanding the regulation of RNAP activity *in vivo*.

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