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Clamping the clamp of RNA polymerase

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Bacterial NusG and its archaeal and eukaryal orthologues Spt5 are the only general transcription factors conserved across the three domains of life. The best studied among them, NusG, is found to be associated with the majority of the transcribed genes in the genome (with its paralogue RfaH picking up the slack) (Belogurov *et al*, 2009), and is implicated in regulating the lateral mobility of RNA polymerase (RNAP) (Bar-Nahum *et al*, 2005; Herbert *et al*, 2010), transcription termination and anti-termination (Nudler and Gottesman, 2002), coordinating transcription and translation (Burmann *et al*, 2010; Proshkin *et al*, 2010), and silencing horizontally transferred genes (Cardinale *et al*, 2008). Understanding of the mechanism of these factors requires detailed structural information about the complexes they are a part of, most importantly that of transcription elongation. Several high-resolution structures of proteins from this family are available, but until now the attempts to co-crystallize NusG or Spt5 with its primary target, RNAP, have failed. Two recent works from the labs of Murakami (Klein *et al*, 2011) and Cramer (in this issue of *The EMBO Journal*) succeeded in circumventing the problems that plagued the conventional strategies.

Klein *et al* utilized a hybrid approach by obtaining a high-resolution structure of archaeal (*Pyrococcus furiosus*) Spt4/5 factor and manually fitting it into a low-resolution cryo-EM density map of the Spt4/5–RNAP complex. In the absence of a RNAP structure from *P. furiosus*, the *Sulfolobus sulfataricus* structure was fitted into the same density map. The resulting rigid body model placed the Spt5-NGN (NusG N-terminal) domain near the clamp helices/coiled-coil motif of the RNAP, which is also the major binding site for NusG, RfaH, and σ initiation factors.

The same clamp helices domains were the target of the co-crystallization efforts, as reported in this issue of *The EMBO Journal* by Cramer and colleagues. Using the structure of the yeast pol II as a template, Martinez-Rucobo *et al* (2011) engineered a minimal clamp helices domain by splicing together fragments from the largest subunits of *P. furiosus* RNAP with short peptide linkers. This ingenuity was well rewarded when this recombinant domain (rClamp) co-crystallized with the Spt4/5. Consistent with the findings of Klein *et al*., the interface between the clamp domain and Spt4/5 consisted largely of the clamp coiled coil and hydrophobic concave patch on the surface of Spt5. The biochemical data available for NusG and RfaH are consistent with the Spt5:rClamp binding mode, indicating that the mode of NGN domain recruitment to the RNAP is essentially conserved among bacteria, archaea, and eukarya. This in turn allowed Martinez-Rucobo *et al*. to model bacterial (*Thermus thermophilus*) RNAP in a binary complex with NusG, where the mechanistic implications of this work are the most conspicuous: the NGN domain anchored by the clamp coiled coil reaches over the RNAP active site cleft towards the so-called

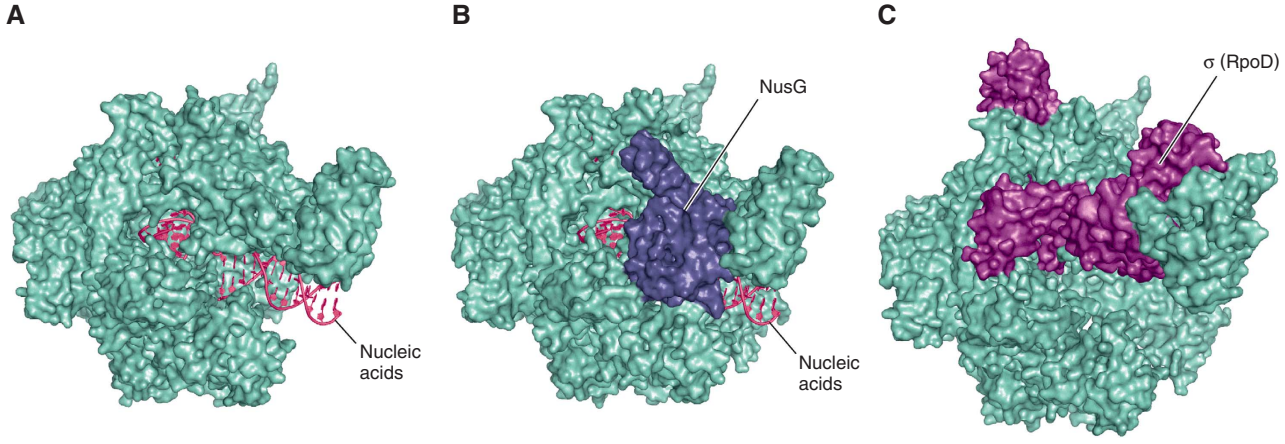

Figure 1 Binding of transcription factors to the clamp region of RNA polymerase. (**A**) Factor-free *T. thermophilus* elongation complex. (**B**) NusG-bound *T. thermophilus* elongation complex. (**C**) *T. thermophilus* RNA polymerase holoenzyme. Core RNA polymerase—green-cyan surface, NusG—purple–blue surface, σ (RpoD)—purple surface, nucleic acids—hot-pink cartoon.

lobe and protrusion elements on the other side, effectively closing the cleft (Figure 1).

The only caveat with using the structure of archaeal clamp/NGN co-crystal as a template for modelling their bacterial or eukaryal counterparts is that the latter do not efficiently bind to a free RNAP. This was rectified by modelling the complete yeast pol II elongation complex (EC). Here the NGN domain of Spt5 separates the downstream and upstream portions of the DNA, locking the RNA–DNA hybrid and the non-template strand in the active site cleft. It was demonstrated biochemically nearly 10 years ago that the NusG paralogue, RfaH, binds to a specific sequence exposed as the non-template strand in the EC (Artsimovitch and Landick, 2002); yet, the details of this interaction remained obscure; remarkably, the model of yeast EC bound by Spt5 NGN, constructed by Cramer and colleagues, revealed a potential interaction between NGN and the non-template DNA in the transcription bubble. Apparently, this interaction could also be broadly conserved among the NusG/Spt5 factors in all three domains of life, as well as among their specialized paralogues. It is also worth noting that structurally and evolutionarily unrelated bacterial initiation factor σ interacts with EC to induce the σ-dependent pausing by forming contacts with the clamp domain coiled coil and the non-template strand of DNA in the transcription bubble (Ring *et al*, 1996). Together, these findings indicate that simultaneous contacts with the clamp domain and the non-template DNA can be instrumental for modulating the pausing behaviour of RNAP.

Cramer and colleagues note that NGN domain binding to the EC completes the encirclement of the nucleic acids partially achieved by the RNAP (Figure 1); the same finding was likened by Klein *et al*. to the replisome sliding DNA clamp and the ring-like architecture of the processive helicases to explain the impact of Spt4/5 on the RNAP processivity (by preventing the disengagement of DNA from the EC) (Klein *et al*, 2011). In its narrowest sense this processivity is defined as the number of nucleotides incorporated into the nascent RNA before the complex dissociates. However, the stability of the complex already appears to be quite high (an EC *in vitro* is stable for days) and thus not rate-limiting. This high stability is at least in part explained by the fact that some elements of the nucleic acid scaffold are already enclosed in the absence of bound NGN (e.g. the RNA–DNA hybrid in the main channel and RNA in its exit channel). An alternative explanation of the effect of the NGN domain binding on the enzyme processivity lies in the non-covalent link it creates between the mobile clamp domain and the lobe/protrusion elements elsewhere in the RNAP, thereby restricting its mobility and allosterically affecting the rates of translocation, pausing, and arrest.

Allostery is often invoked (even if not explicitly addressed) in transcription research, where an understanding of the RNAP as a conformationally mobile enzyme combining catalytic and mechano-chemical steps in each NTP addition cycle continues to emerge. A recent work by Tagami *et al* (2010) allowed a glimpse into the translocation cycle of the bacterial EC, linking the concept of the Brownian ratchet in transcription (Bar-Nahum *et al*, 2005) with the actual ratcheting of the two rigid modules (core and shelf) of the RNAP. Remarkably, the pivoting of these modules, each about a thousand amino acids, occurs around a mere three peptide bonds, which probably explains the ease with which ratcheting occurs in either direction, with only the thermal energy as the input (Tagami *et al*, 2010). Hence, any perturbations of this equilibrium by additional restraints on free ratcheting could greatly impact the enzyme translocation in either direction. The confidence of the current NGN-binding models may not be sufficiently high as to warrant an exploration of NusG/Spt5's effects on the conformational dynamics of the EC by computational methods, but as more high-resolution structural data become available, an application of molecular dynamics or coarse-grained methods could provide insights into their mechanisms, which are unobtainable by examination of the static structures.

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Conflict of interest

The authors declare that they have no conflict of interest.

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