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Structure and function of RapA: A bacterial Swi2/Snf2 protein required for RNA polymerase recycling in transcription

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Abstract

One of the hallmarks of the Swi2/Snf2 family members is their ability to modify the interaction between DNA-binding protein and DNA in controlling gene expression. The studies of Swi2/Snf2 have been mostly focused on their roles in chromatin and/or nucleosome remodeling in eukaryotes. A bacterial Swi2/Snf2 protein named RapA from *Escherichia coli* is a unique addition to these studies. RapA is an RNA polymerase (RNAP)-associated protein and an ATPase. It binds nucleic acids including RNA and DNA. The ATPase activity of RapA is stimulated by its interaction with RNAP, but not with nucleic acids. RapA and the major sigma factor $\sigma 70$ compete for binding to core RNAP. After one transcription cycle *in vitro*, RNAP is immobilized in an undefined posttranscription/posttermination complex (PTC), thus becoming unavailable for reuse. RapA stimulates RNAP recycling by ATPase-dependent remodeling of PTC, leading to the release of sequestered RNAP, which then becomes available for reuse in subsequent cycles of transcription. Recently, the crystal structure of RapA that is also the first full-length structure for the entire Swi2/Snf2 family was determined. The structure provides a framework for future studies of the mechanism of RNAP recycling in transcription.

Keywords

RapA; bacterial Swi2/Snf2; RNA polymerase recycling; structure of Swi2/Snf2; transcription complex remodeling

Introduction

The Swi2/Snf2 family proteins consist of a large number of subfamilies of eukaryotic, archaeal and bacterial origins [1]. The most widely studied Swi2/Snf2 proteins are eukaryotic members and form multi-subunit complexes; some of them are described in this special issue. ATP-dependent chromatin and/or nucleosome remodeling by the Swi2/Snf2 complexes is known to be an important aspect of transcriptional regulation in eukaryotes. The eukaryotic Swi2/Snf2 makes DNA tightly packaged in nucleosomes more accessible for RNAP and transcription factors, including both positive and negative regulatory proteins.

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In contrast to eukaryotic chromosomes, which are fully packaged by histones into nucleosomes and chromatin fiber [2, 3] with an average protein/DNA mass ratio of ~1:1, the bacterial chromosome, named the nucleoid, is only partially covered by nucleoid-associated proteins with a canonical ratio of ~1:10 [4]. The difference in chromosomal organizations between the prokaryotes and eukaryotes implies that bacterial Swi2/Snf2 has a different target and mechanism than its eukaryotic counterparts. In addition, bacterial Swi2/Snf2, of which RapA is a representative, works alone, unlike its eukaryotic counterpart whose functional form in general is a multi-subunit complex.

RapA, which is sometimes referred to as HepA in the literature in *E. coli*, is so far the best characterized bacterial Swi2/Snf2 [5–8]. In this mini review, we summarize its history of discovery and investigation, its biochemical properties and role in RNAP recycling during transcription, as well as the regulation of *rapA* and the phenotypes associated with the *rapA* mutants. In addition, important features in the RapA structure are highlighted and a model for PTC remodeling by RapA is presented. The advantage of genetics, biochemistry and physiology of the simple *E. coli* model system will greatly facilitate future studies of the molecular mechanism of this bacterial Swi2/Snf2.

RapA is an RNAP-associated protein and an ATPase

There are two forms of RNAP in *E. coli*. Core RNAP consists of $\alpha_2\beta\beta'\omega$ and holoenzyme ($\alpha_2\beta\beta'\omega\sigma$) has an additional sigma factor [9]. The holoenzyme containing the major sigma factor $\sigma 70$ is the house keeping RNAP for transcription of most of the genes in *E. coli*. During preparations of RNAP, many proteins are co-purified with RNAP [10]. RapA (110-kDa), which is as abundant as $\sigma 70$, is consistently associated with RNAP, and is eluted from the Mono-Q column in fractions immediately after the holoenzyme [11]. The constituents of the RapA-containing fractions are subunits of holoenzyme and RapA. With purified components, apparently stable complexes of holoenzyme-RapA and core RNAP-RapA can be reconstituted in the presence of 0.1–0.2 M NaCl, as analyzed by gel filtration and glycerol gradient ultracentrifugation methods; however, RapA exhibits a higher affinity for core RNAP than holoenzyme with increased concentration of salt [12].

Independently, using similar but modified procedures for RNAP purifications, the 110-kDa protein was also discovered, but was named HepA [13]. However, the protein was found to be co-eluted from the Mono-Q column in fractions of both core RNAP and the holoenzyme; when the interactions of RNAP and the protein were analyzed by electrophoresis of native polyacrylamide gels, it was found that the protein apparently is only associated with core RNAP [13]. The nature of the interaction between RapA and the two forms of RNAP were further analyzed with competition experiments, in which $\sigma 70$ and RapA compete for the binding of core RNAP that is limiting in the presence of 0.3M NaCl [14]. Under the conditions used, $\sigma 70$ competes effectively for the binding of core RNAP, as analyzed by gel filtration methods. Thus it becomes clear that the original identification of the complexes of RapA and holoenzyme [11, 12] is in fact a mixture of holoenzyme and the RapA•core RNAP complex under the conditions when RapA and core RNAP are in excess, reflecting the dynamic interactions of these proteins.

RapA cross-links to the α and β' subunits of RNAP [12], although the interaction sites have not been identified. In the absence of RapA, the α and $\sigma 70$ subunits of RNAP cross-link effectively. In the presence of RapA, however, the efficiency of the cross-linking between the α and $\sigma 70$ subunits is greatly reduced; this result is also consistent with the possibility that RapA competes with $\sigma 70$ for a common binding site on core RNAP. The dynamic competition between RapA and $\sigma 70$ for core RNAP has clear implications for the role of RapA in RNAP recycling during transcription.

Like other Swi2/Snf2 proteins, RapA, as an ATPase, has six conserved functional motifs (motifs I–VI) in the RecA-like architecture of ATPases and helicases [11, 13, 14]. The ATPase activity of RapA is stimulated by its association with RNAP, but not nucleic acids. Based on yeast Swi2/Snf2 mutations [15], mutant RapA proteins with mutations in conserved regions involved in ATP binding, R183A (in motif I), or D280A and E281A (in motif II), are defective in ATPase activity [16]. Similarly, a RapA mutant containing two changes, R599A/Q602A, in proximity to the ATP-binding site, is also defective in ATPase activity [17]. However, another RapA mutant with two mutations close to the ATP-binding site, R221A/ R222A, instead enhances the ATPase activity significantly compared to the wild type [17]; the reason for this enhancement is not clear. RapA is also able to hydrolyze dATP and this activity is also enhanced by RNAP [11]; however, RapA hydrolyzes other NTPs and dNTPs very poorly.

RapA binds to nucleic acids, including single and double strand (ds) RNA and DNA [11], but it possesses no detectable RNA and DNA-dependent helicase activity. RapA was also found to be associated with other RNAs *in vitro* [18]. The binding site for dsDNA in RapA was identified based on structural analysis and modeling [14]; however, the binding sites for other nucleic acids are not known.

Regulation of *rapA* and phenotypes of the *rapA* null mutants in *E. coli*

The expression of RapA is growth phase dependent, peaking at the early log phase when cells are cultured in LB [19]. Furthermore, *rapA* is regulated by growth rate, similar to the regulation of rRNA synthesis and other stringently controlled genes [20, 21]. As expected, the interaction of RNAP and the *rapA* promoter is intrinsically unstable and is sensitive to salt and supercoiling [19], exhibiting the hallmark of the stringent promoters [22]. Under different physiological conditions, RapA is highly expressed in biofilms, but only minimally in planktonic cells [23]. However, it is not clear how the regulation of *rapA* is related to the function of RapA under different growth environments in the cell.

RapA is not essential under normal growth conditions; there are only a few phenotypes that are associated with the *rapA* null mutant. First, the growth of the *rapA* mutant is severely retarded without affecting the efficiency of plating compared to the wild type when cells were plated on LB agar plates containing high concentrations of salt [16, 17]. Intriguingly, the growth defect of the *rapA* mutant is specific to LB agar plates, no difference in growth rate is seen between the wild type and the *rapA* mutant when cells are cultured in liquid LB with high concentrations of salt. These results suggest that RapA is important for osmotic stress response [24, 25] only when cells are grown in solid media. Bacterial physiology is clearly different when cells are grown in solid phase rather than in liquid media. The reason for the phenotype of the *rapA* mutant is unknown. Transcriptional profiling of the wild type and *rapA* mutant under the specific stress condition may provide some insights.

Another phenotype is that the *rapA* mutant decreases antibiotic resistance in biofilms compared to the wild type [23]. Analysis of global gene expression patterns between the wild-type and *rapA* mutant biofilms indicates that RapA is required for the expression of a putative multidrug resistant pump and a cell wall-related function in biofilms. How RapA controls the expression of these genes in biofilms remains to be elucidated. Note that biofilm is also a solid-phase-related phenotype. In addition, although disruption of the *rapA* gene was initially reported to cause UV sensitivity of the cell [13], further studies failed to confirm this phenotype of the *rapA* mutant; moreover, the *rapA* mutant has no significant effect on mutation rate [12]. Together, the phenotypes of the *rapA* mutants suggest that RapA is important under some special stress of physiological environments, but it is

unlikely to be involved directly in DNA repair. The role of RapA in bacterial stress response needs further investigation.

RapA activates transcription by promoting RNAP recycling

Compared with *in vivo* studies of RapA, much progress has been made in the biochemical analysis of RapA *in vitro*. Since the identification of RapA [11, 13], numerous efforts have been made to study the function of RapA in transcription assays *in vitro*; however, the results were initially unclear. An important clue was suggested when it was found that RapA activates transcription in a multiple-round transcription assay, but has no effect in a single-round transcription assay; the activation of transcription requires the ATPase activity of RapA [16]. As illustrated in Fig. 1, RapA greatly stimulates transcription only in the absence of DNA competitor heparin or re-initiation inhibitor rifampicin. These results are an indication of RNAP sequestration on DNA and/or RNA in an undefined posttranscription/posttermination complex (PTC) after a single round of transcription. A model was proposed that the PTC is remodeled by RapA, which enables RNAP to enter a new cycle of transcription [16]. In this model, the key role of RapA is to mobilize and/or release RNAP from the PTC, enabling RNAP recycling. Other models, based on the results that RapA interacts with RNA [18], have proposed that RapA mediates redistribution of RNA during *in vitro* transcription [17]; however, the nature of RapA-RNA interactions and its relevance to the function of RapA remain to be determined. In addition, transcription activation by RapA requires a relatively high concentration of salt in the reaction [16]. The mechanism for this requirement is not clear; however, it has been established that the thermodynamics and kinetics of interaction between transcription factors and nucleic acids are weakened with increasing concentration of salt [26, 27].

A kinetic template-switching assay is performed to monitor the rate of RNAP sequestration during transcription and to test if RapA promotes the release of sequestered RNAP from the PTC [14]. In this assay, transcription is carried out on two DNA templates sequentially. As depicted in Fig. 2, RNAP becomes rapidly sequestered during transcription of the first template containing the *pTac* promoter and thus unavailable for binding to the second λP_L template that is added to the reaction after a short (1–2 min) delay. Addition of RapA dramatically stimulates utilization of the second λP_L template added even after a much longer (5 min) delay. This experiment demonstrates that RapA promotes transfer of RNAP *in trans* from the PTC on the *pTac* template to the λP_L template. Thus, like other Swi/Snf proteins, RapA plays a role in mobilization of nucleic acid-protein complex to facilitate gene expression. However, in contrast to other Swi2/Snf2 proteins, RapA targets the sequestered RNAP directly in the absence of any nucleoid-associated proteins, providing a new paradigm of transcription regulation by transcriptional activators.

On the basis of all the findings, a model is derived to illustrate the action of RapA in the transcription cycle (Fig. 3), which adds three new steps to the exit from the transcription cycle [28]: (a) Core RNAP is sequestered in the PTC after completion of single-round transcription; the PTC could be either an RNAP•DNA binary complex or a RNAP•DNA•RNA tertiary complex; (b) The RapA ATPase releases binary complex RapA•Core RNAP from the PTC; (c) The $\sigma 70$ subunit displaces RapA from the RapA•Core RNAP complex, resulting in the formation of holoenzyme ($\sigma 70$ •Core) capable for recognition of promoters. This process is repeated multiple times and leads to activation of transcription. This model provides a working hypothesis for future studies of the mechanisms by which RapA recycles RNAP in transcription. Moreover, the mode of action of RapA revealed *in vitro* is also useful to identify the potential RapA function in the cell. For example, it is likely that the RapA activity becomes critical under conditions where the

amount of RNAP is limiting in the cell under some stress environments. A challenge in future studies is to identify these stress conditions genetically and/or physiologically.

Structure of RapA provides clues to the mechanism of PTC mobilization

The crystal structure of full-length RapA from *E. coli* at 3.2-Å resolution reveals a seven-domain architecture of the molecule [14]. This is also the first structure of any full-length Swi2/Snf2 protein [29]. As depicted in Fig. 4, the RapA structure contains an N-terminal domain (Ntd), two RecA-like domains (1A and 2A), two Swi2/Snf2-specific domains (1B and 2B), a Spacer domain, a C-terminal domain (Ctd), Linker1 between Ntd and 1A, Linker2 between 1B and 2B, and Linker3 between 2B and Spacer. The Ntd contains two subdomains, NtdA and NtdB, both folded as a highly bent antiparallel β -sheet. The functional roles of the Ntd homologs in other proteins (For details, see [14]) suggest that the Ntd of RapA interacts with both nucleic acids and RNAP. The Spacer and the Ctd are of the α/β fold in nature with a central β -sheet flanked by helices and loops. Structural comparison indicates that the folds of both the Spacer and the Ctd are novel [14]. The functional roles of these two new folds remain to be elucidated. The three linkers contain 31, 25 and 44 amino acid residues, respectively. Linker1 connects the Ntd and the ATPase core; Linker2 tethers the two lobes of the ATPase core; and Linker3 forms an elongated, L-shaped connector between the ATPase core and the Spacer. Together, Linker1 and Linker3 cover a distance of approximately 100 Å across one face of the RapA molecule.

Domains 1A and 2A form the RecA-like architecture that contains the six conserved functional motifs (I–III in domain 1A, IV–VI in domain 2A). The locations of the six motifs in RapA are defined by structure-based sequence alignment with three other RecA-like architectures (Fig. 5). Note that as for the sequence conservation of conserved motifs, those (I, II, V, and VI) mainly for ATP binding show relatively good sequence conservation, whereas those (III and IV) mainly for DNA binding do not. The RecA-like architecture (1A and 2A) and the two Swi2/Snf2-specific domains (1B and 2B) of RapA form the ATPase core, typical for ATPase-dependent Swi2/Snf2 proteins and dsDNA translocases. *In vitro*, RapA binds both single and double strand polynucleotide molecules, but exhibits higher affinity for dsDNA [11]. Superposition of the RecA-like architecture in the RapA structure with those in other helicase-ATP and Swi2/Snf2-dsDNA structures suggests a model for the RapA•ATP•dsDNA complex (Fig. 6). Both the binding mode and the transporting direction of DNA in the model are in agreement with the unified mechanism for Swi2/Snf2 enzymes and DExx box helicase proteins [30]. The RapA•ATP•dsDNA model suggests that the minor groove of dsDNA interacts with domains 1A, 2A, and 2B of RapA (Fig. 6). It has been shown that mutations in domain 2B of a Swi2/Snf2 protein interfere with its catalytic activity [31]. Thus, both structural and biochemical data indicate the involvement of domain 2B in the translocation of Swi2/Snf2 proteins, including RapA, on dsDNA. The model also suggests ATP- and dsDNA-interacting regions/residues of RapA. Mutational analysis of the protein at these implicated regions will be critical in determining the functions involved. A detailed understanding of these regions, however, requires a structure of the RapA•ATP•dsDNA complex.

From the structure of RapA, it is still not clear at this time what the mechanism is underlying the competition between RapA and $\sigma 70$. It is plausible that RapA and $\sigma 70$ may share a common binding site or have partially overlapped binding sites on RNAP. Equally plausible, however, is that binding of $\sigma 70$ to the core RNAP results in conformational changes of the core that preclude binding of RapA. Further structural and mutational analysis of RapA will further our understanding on this important issue.

Summary and perspective

RapA remodels the PTC to promote the release of immobilized RNAP, enabling the reuse of RNAP after one cycle of transcription; the function of RapA depends on ATP binding and hydrolysis. The proposed mode of RapA action is supported by the structure of RapA and is consistent with the general mechanisms of Swi2/Snf2 in remodeling various complexes of transcription factors and nucleic acids. The nature of the PTC, however, needs to be determined. Investigations on why RNAP is sequestered in the PTC after one round of transcription will shed light on the mechanism of RapA. Studying the dynamic interaction between RapA and $\sigma 70$ in transcription cycles will provide insight into the mechanism of transcription. The research on RapA will be facilitated with additional RapA structures at higher resolutions and the structures of RapA•dsDNA, RapA•core RNAP and other complexes. Finally, challenges remain in the identification of the function of RapA in *E. coli* genetics and physiology.

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Figure 1. RapA activates transcription only under conditions enabling multiple cycles of transcription

The reaction included RNAP and a plasmid DNA containing *pTac* either in the presence (+) or absence (-) of RapA; *in vitro* transcription assay was performed as described [16]. Transcript from the *pTac* promoter is indicated. When indicated, heparin or rifampicin was added in the reaction. Both inhibitors block multiple-round transcriptions because heparin titrates out unbound RNAP and rifampicin prevents reinitiation. The inhibitory effect of heparin on RapA-mediated transcriptional activation was previously reported [16].

Figure 2. RapA facilitates the release of RNAP in an *in vitro* DNA template-switching transcription assay

The reaction was started (at time 0) by the addition of NTPs into a preincubated complex of RNAP and a plasmid DNA containing *pTac* (the first template) either in the presence (+) or absence (-) of RapA; at various times as indicated, an equal molar amount of another plasmid DNA containing λP_L (as a second template) was added, followed by 1 hr incubation for the reaction to complete. The transcripts for *pTac* and λP_L are indicated. The *RNAI* transcript originates from a relatively weak promoter present in both plasmids is also indicated (Adapted from the study by Shaw et al [14]).

Figure 3. Model for the RapA-mediated RNAP recycling in transcription

RNAP holoenzyme ($\sigma 70$ -Core) binds to a promoter of DNA template to initiate transcription. The $\sigma 70$ subunit is released shortly after initiation. After elongation and termination, a posttranscription/posttermination complex (PTC) is formed, in which RNAP becomes sequestered. Using its ATPase activity, RapA remodels the PTC, leading to the release of the RapA-Core complex. The $\sigma 70$ subunit competes with RapA and displaces it to form $\sigma 70$ -Core (holoenzyme), which is able to start another cycle of transcription. The repeated process enables RNAP recycling in transcription. This model, based on recent works [14], is evolved from the previous one [16]

Figure 4. Crystal structure of full-length RapA from *E. coli*

On the top, the domain organization of RapA is shown with boundaries (residue numbers in the sequence). In the middle, the domain arrangement is shown schematically by two views of the molecule related to each other by a 180° rotation around the vertical axis. On the left, right and bottom, the folds of individual domains and a cluster of four domains (1A, 1B, 2A, and 2B, which form the ATPase core of the enzyme) are illustrated. The Ntd (N-terminal domain) contains two sub-domains, NtdA and NtdB, which share the same fold. The Linker3 is illustrated as a stick model (C, grey; N, blue; O, red) outlined with the composite annealed omit map (green net, 2Fo - Fc, contoured at 1.0 σ). The Spacer and the Ctd (C-terminal domain) exhibit novel folds. The sulfate ion bound to the ATPase core is shown as a sphere model (Adapted from the study by Shaw et al [14]).

Figure 5. RecA-like architecture and the six conserved functional motifs

(A–D) The RecA-like architecture of DExx box helicase PcrA in complex with DNA and an ATP analog (PcrA•ATP•DNA, PDB entry 2PJR), ZfRad54 in complex with a sulfate ion (ZfRad54•SO₄, PDB entry 1Z3I), SsRad54 in complex with dsDNA (SsRad54•DNA, PDB entry 1Z63), and *E. coli* RapA in complex with a sulfate ion (RapA•SO₄, PDB entry 3DMQ) are illustrated as Ca traces on the basis of the superposition of domain 1A. Domain 1A is shown in blue and 2A in yellow. Indicated in red are the six functional motifs, the sequences of which are shown in panel E. The ATP and SO₄ are shown as stick models while DNA molecules as tubes. (E) Structure-based sequence alignment for the six functional motifs in PcrA•ATP•DNA, ZfRad54•SO₄, SsRad54•DNA and RapA•SO₄. Amino acid residues highlighted in red are also shown in red in panels A–D. The functional motifs are labeled in panel D only. (Adapted from the study by Shaw et al [14])

Figure 6. Model of the RapA•ATP•dsDNA complex

The model suggests a role of domain 2B in ATP-binding-associated advancement of dsDNA for Swi2/Snf2 proteins. In this view, the ATP molecule is not visible. The white arrow suggests the transporting direction of DNA; the black arrow suggests the translocation direction of the ATPase core along the minor groove of dsDNA. For clarity, only the ATPase core is shown instead of the full-length structure (Adapted from the study by Shaw et al [14]).