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Crystal Structure of *Escherichia coli* Rnk, a New RNA Polymerase-Interacting Protein

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Sequence-based searches identified a new family of genes in proteobacteria, named *rnk*, which shares high sequence similarity with the C-terminal domains of the Gre factors (GreA and GreB) and the *Thermus*/*Deinococcus* anti-Gre factor Gfh1. We solved the X-ray crystal structure of *Escherichia coli* regulator of nucleoside kinase (Rnk) at 1.9 Å resolution using the anomalous signal from the native protein. The Rnk structure strikingly resembles those of *E. coli* GreA and GreB and *Thermus* Gfh1, all of which are RNA polymerase (RNAP) secondary channel effectors and have a C-terminal domain belonging to the FKBP fold. Rnk, however, has a much shorter N-terminal coiled coil. Rnk does not stimulate transcript cleavage *in vitro*, nor does it reduce the lifetime of the complex formed by RNAP on promoters. We show that Rnk competes with the Gre factors and DksA (another RNAP secondary channel effector) for binding to RNAP *in vitro*, and although we found that the concentration of Rnk *in vivo* was much lower than that of DksA, it was similar to that of GreB, consistent with a potential regulatory role for Rnk as an anti-Gre factor.

Keywords: Rnk; RNA polymerase-interacting protein; X-ray crystallography

Introduction

Gre factors (GreA and GreB)[1] promote transcription elongation in bacteria by stimulating the intrinsic endonucleolytic transcript cleavage activity of the RNA polymerase (RNAP).[2] Gre factors are required for the natural progression of RNAP *in vivo*.[3,4] In addition to rescuing arrested complexes and increasing the overall elongation rate,[5] Gre factors may play a role in modulating RNAP behavior at pause signals,[3] increasing transcriptional fidelity,[6] and stimulating promoter clearance.[7–9]

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Abbreviations used: Rnk, regulator of nucleoside kinase; RNAP, RNA polymerase; NTD, N-terminal domain; CTD, C-terminal domain; *Tth*, *Thermus thermophilus*; *Taq*, *Thermus aquaticus*; *Ec*, *Escherichia coli*; TEC, ternary elongation complex; PDB, Protein Data Bank; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin.

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Gre factors comprise two structural/functional domains.[10] The N-terminal domain (NTD) consists of a 60-Å-long coiled coil that extends into the RNAP secondary channel to the RNAP catalytic site and is critical for stimulating transcript cleavage activity.[11–13] The globular C-terminal domain (CTD) consists of a β-sheet flanked by a small α-helix.[10–12] The CTD, which is important for binding RNAP, interacts with the β′ subunit of RNAP at the entrance of the RNAP secondary channel.[10–13] Biochemical and structural data have converged to a model whereby conserved acidic residues at the Gre coiled-coil tip stabilize the binding of the second Mg^{2+} ion in the RNAP active site required for the endonucleolytic cleavage reaction.[13–16]

Similar to the Gre factors, DksA is organized into two structural domains. It has a long coiled coil similar in structure to that of the Gre factors, but its second domain is distinct and contains a Zn-finger motif.[17] Like the Gre factors, DksA binds to RNAP with its coiled-coil structural element placed directly in the RNAP secondary channel[17,18] (I. Toulokhonov and R.L.G., unpublished data). DksA is essential for control of rRNA promoters at all times in bacterial growth, including following nutrient starvation, when it cooperates with the alarmone ppGpp to regulate expression from many operons during the stringent response.[19,20] Recent studies have shown that GreB can fulfill some roles of DksA *in vitro*, allowing rRNA promoters to sense changes in the concentrations of ppGpp and the first NTP in the transcript when the *dksA* gene is deleted.[21]

In addition to a GreA homolog, the hyperthermophiles *Thermus thermophilus* (*Tth*) and *Thermus aquaticus* (*Taq*) possess another Gre factor homolog (Gfh1) that lacks transcript cleavage activity and competes with GreA for RNAP binding.[22] This anti-Gre factor contains domains structurally similar to that of GreA.[10,23–25] Crystal structures of *Taq* and *Tth* Gfh1 revealed a large conformational change compared with *Escherichia coli* (*Ec*) GreA, affecting the relative orientation of the coiled coil and the CTDs. Biochemical data suggested that Gfh1 binds RNAP in a conformation similar to that observed for the Gre factors but that it switches to an alternate conformation at high pH.[10,13,25] Conserved interdomain contacts that may stabilize the GreA-like conformation of Gfh1 were revealed by modeling Gfh1 in its GreA-like conformation.[23]

Our database searches for Gre factor/Gfh1 homologs revealed another family of proteins in proteobacteria, known as regulator of nucleoside kinase (Rnk), which shares substantial sequence similarity with the Gre and anti-Gre factors. Mutations in *rnk* drastically reduce the level of nucleoside diphosphate kinase in *Ec*, which is an important enzyme involved in maintaining cellular NTP and dNTP pools.[26] By an unknown mechanism, *Ec* Rnk is also a multicopy suppressor of an alginate-deficient phenotype in *Pseudomonas aeruginosa* caused by deletion of *algQ* (also called *algR2*[27]).

We present here the 1.9-Å-resolution crystal structure of *Ec* Rnk. The structure reveals a globular CTD that is structurally conserved with the Gre and anti-Gre factors but an NTD containing a much shorter coiled coil. The CTD belongs to the FKBP fold first identified in the immunophilin family.[28] The FKBP fold has been found in proteins with a wide variety of functions, from a peptidyl–prolyl isomerase to proteins that make protein/protein interactions within large molecular assemblies. Structural features of the Gre/Gfh1/Rnk FKBP domain show that it belongs to the latter group. We further demonstrate that *Ec* Rnk competes for binding and function with the RNAP secondary channel effectors GreA, GreB, and DksA *in vitro*. Unlike Gre factors,[10,11] however, Rnk does not cross-link with the 3′-end of the RNA transcript in the RNAP ternary elongation complex (TEC), stimulate transcript cleavage, or decrease the lifetime of the RNAP–promoter complex.[21] The cellular concentration of Rnk in both log and stationary phases is comparable to that of GreB and only a few-fold lower than that of GreA, suggesting that Rnk might compete with, and thereby regulate, Gre factor activity *in vivo*. We found that Rnk is dispensable for regulation of rRNA transcription initiation *in vivo*, consistent with its much lower cellular concentration than DksA. However, we also were unable to detect effects of Rnk on Gre factor function in transcription from the *tnaC* promoter *in vivo*. Our results thereby suggest a tantalizing, although unconfirmed, role for Rnk *in vivo* as an anti-Gre factor.

Results

Identification of Rnk proteins as a new family of Gre factor homologs

The anti-Gre factor Gfh1 has only been found in the *Thermus/Deinococcus* phylum. Searching for Gfh1 homologs using the program Ballast,[29] we found proteins sharing significant sequence similarity in a region covering the CTD (residues 79 to 151) of *Taq* Gfh1. The Gfh1 homologs belong to the nucleoside diphosphate kinase regulator family (Rnk), with the closest homolog from *Geobacter sulfurreducens* (expectation value, 1×10^{-9}). In an inverse search using *Ec* Rnk, elongation factors were found immediately after Rnk family members (elongation factor Q82T10_niteu: expectation value, 1×10^{-28}; ballast rank, 11; blast rank, 19).

During our search, we found that the Rnk proteins seem to be limited to Gram-negative proteobacteria (Fig. S1). *Ec* Rnk (sw:P40679) is a 136-amino-acid protein that shares significant sequence identity with *Taq* Gfh1: 22.79% identity between the full-length proteins and 28.9% identity over their CTDs (*Ec* Rnk residues 48 to 136, *Taq* Gfh1 residue 79 to the C-terminus). By comparison, *Ec* Rnk shares 22.06% sequence identity with *Ec* GreA and 15.44% with *Ec* GreB (Gfh1 shares 25% identity with *Ec* GreA). The N-terminal residues of Rnk (residues 1 to 47) do not exhibit significant sequence similarity with the Gre factor NTDs (residues 1 to 76).

Secondary-structure prediction for Rnk using PHD[30] revealed that the CTD of Rnk possesses