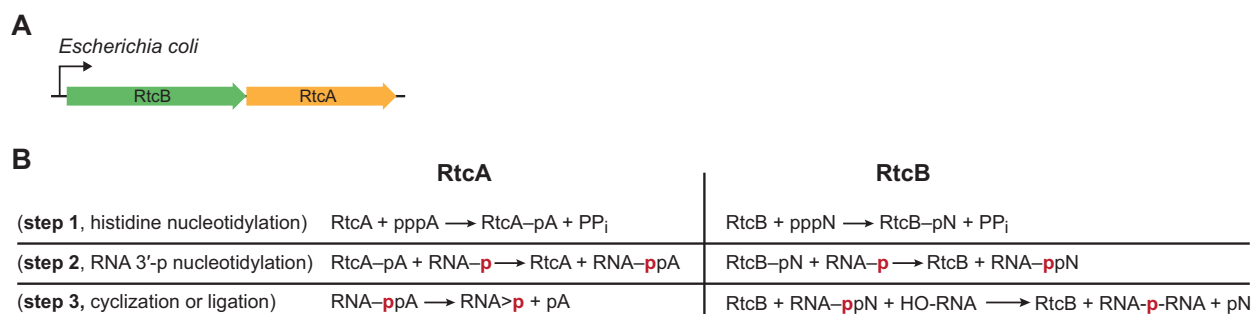
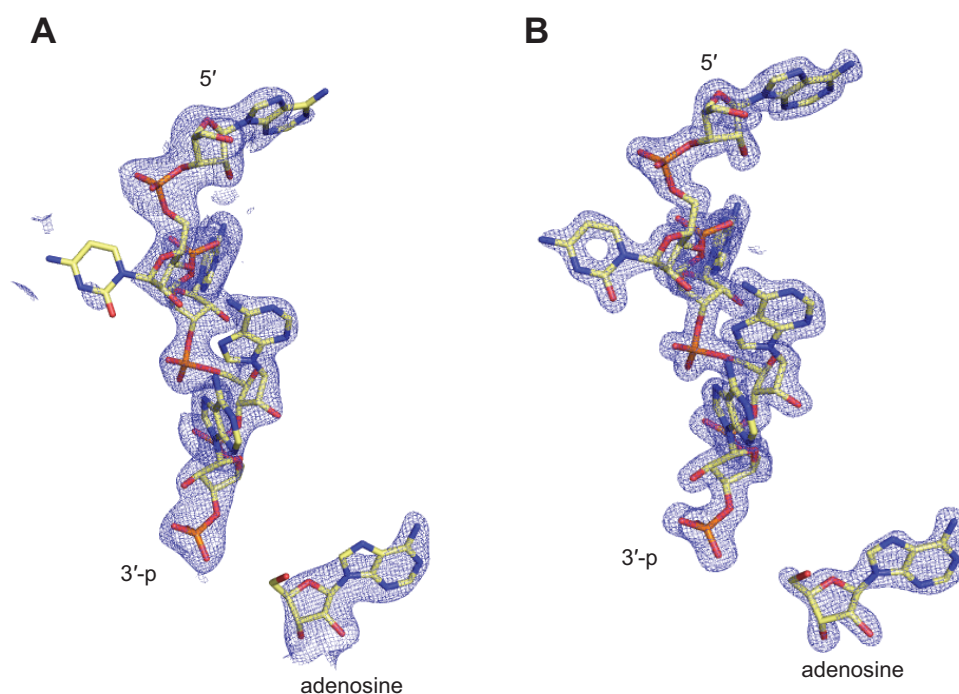


## Structure of RNA 3'-phosphate cyclase bound to substrate RNA

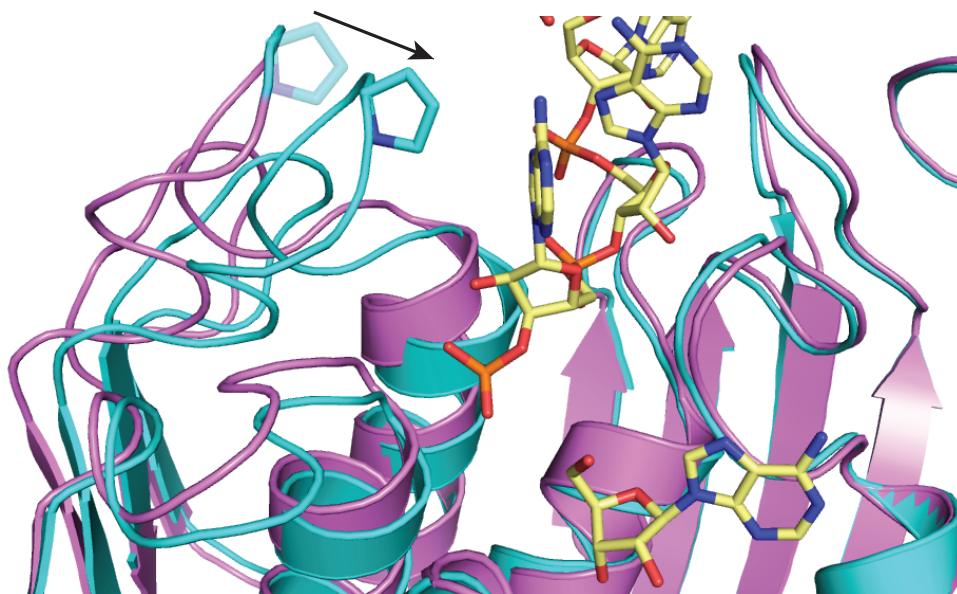
Kevin K. Desai, Craig A. Bingman, Chin L. Cheng, George N. Phillips, Jr. and Ronald T. Raines



**SUPPLEMENTAL FIGURE S1.** Comparison of RtcA and RtcB. (A) The operon organization of *rtcA* and *rtcB* in *Escherichia coli*. (B) Comparison of the three-step reaction mechanisms of RtcA and RtcB. For *Pyrococcus horikoshii* RtcB catalysis, pppN is GTP in the absence of Archease. In the presence of Archease, pppN can be ATP, GTP, dGTP or ITP. Despite the similarity in the first two reaction steps catalyzed by RtcA and RtcB, the enzymes share no amino acid sequence identity.



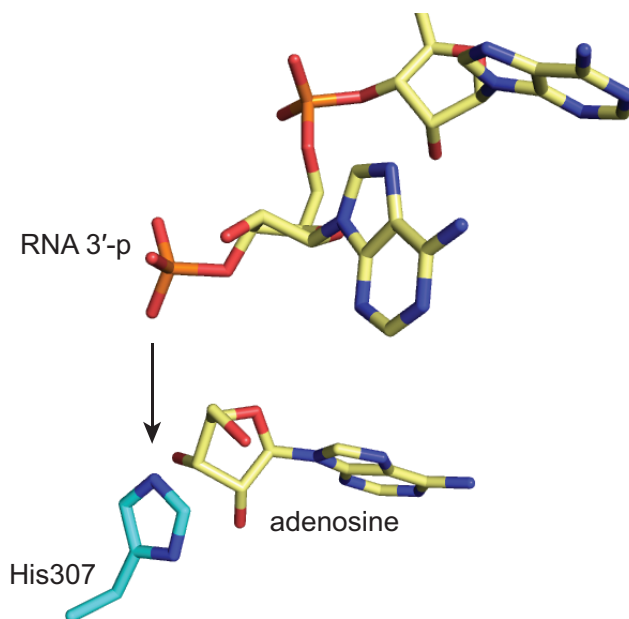
**SUPPLEMENTAL FIGURE S2.** Electron density maps of the RNA and adenosine (represented as sticks) bound to RtcA subunit B. (A) Composite simulated annealing omit map (blue mesh) contoured at  $1.2\sigma$ . (B) Electron density (blue mesh,  $2F_o - F_c$ ) of the refined model contoured at  $2.0\sigma$ .



**SUPPLEMENTAL FIGURE S3.** Superposition of apoRtcA (magenta) and RtcA-RNA (teal) depicting the conformational change of the loop comprising residues 37–42. The maximal C<sup>α</sup> displacement is 5.1 Å at Asn38. In addition, Pro40 moves 3.6 Å upon RNA binding to make van der Waals contact with the A5 nucleobase.

<i>H. sapiens</i>	-MAGPRVEVDGSIMEGGGQILRVSTALSCLLGLPLRVQKIRAGRSTPGLR	49
<i>D. melanogaster</i>	MDAEKMLEIDGSYLEGGGQALRNALSLSLSCILGKPVRRVVKIRASRPSGLS	50
<b><i>P. horikoshii</i></b>	-----MITIDGSYEGGGQILRTSVALSTITGEPVRRVNRANRPNGLR	<b>45</b>
<i>E. coli</i>	--MKRMIALDGAQEGGGQILRSALSLSMITGQPFTTITSIRAGRAKPGLL	48
<i>H. sapiens</i>	POHLSGLEMIRDLCDGQLEGAEIGSTEITFTPEKIKGGIHTADTKTAGSV	99
<i>D. melanogaster</i>	HOHLHGLNLLRDITNADVGNLYLLSSTVEFTPRITILDNTYRVETHTAASI	100
<b><i>P. horikoshii</i></b>	<b>POHLHAILALKHLANA EVKGAHVGSREL VFI PKKLEAKEISIDIGTAGSI</b>	<b>95</b>
<i>E. coli</i>	ROHLTAVKAATEICGATVEGAELGSQRLFRPGTVRGGDYRFAIGSAGSC	98
<i>H. sapiens</i>	CLLMQVSMPCVLF AASPSELHLKGGTNAEMAPQIDYTMVMVFKPIVEKFGF	149
<i>D. melanogaster</i>	TLIYQMALPVLLFAGRPSRLIVSGGTNVDFAAPPVEYMQEVLNPKHFGV	150
<b><i>P. horikoshii</i></b>	<b>TLVLQALLPAMVFAREKVKFRITGGTDVSWSPVDYLSNVTLFALEKIGI</b>	<b>145</b>
<i>E. coli</i>	TLVLQTVLPALWFADGSPRVEVSGGTDNPSAPPADFIRRVLPELLAKIGI	148
<i>H. sapiens</i>	IFNCDIKTRGYYPKGGGEVIRMSPVKQLNPINLTERGCVTKIYGRAFVA	199
<i>D. melanogaster</i>	SFDLKVQRYGFYPRGQRCQLDVQPVTKLNSGKLVAFGRIKSVSGVAYCA	200
<b><i>P. horikoshii</i></b>	<b>HGEIRVIRRGHYPKGGGIVEGYVEPWNEKRELVAKESRIKIEGISHAT</b>	<b>195</b>
<i>E. coli</i>	HQQTTLRHGFYPAGGGVVATEVSPVASFNTLQLGERGNIVQMRGEVLLA	198
<i>H. sapiens</i>	GVLPPFKVAKDMAAAVRCIRKEIRDLYVNIQPVQEPKDQAFNGNGI III	249
<i>D. melanogaster</i>	GRLPVNIAIDMQQTAQREIHRWLWPSQQCSIEPIKHSRQKAFHNGAGILMT	250
<b><i>P. horikoshii</i></b>	<b>N-LPSHVAERQARA AKDELLQLKVP IEIRTE-----ISRSIGPGSGIVVW</b>	<b>239</b>
<i>E. coli</i>	G-VPRHVAEREIATLAGSFSLSHEQNIHN-----LPRDQGPNTVSLE	239
<i>H. sapiens</i>	AETSTGCLFAGSSLGKRGVNADKVGIEAAEMLLANLRHGGTVDEYLQDQL	299
<i>D. melanogaster</i>	VNTTSDVVLGASALGKKRIDGHVVGSEASCQLGDYMRKQVCVDDYMQDQL	300
<b><i>P. horikoshii</i></b>	<b>AETDC-LRLGGDALGKKGKPAEIVGKEAAQELLDQLKPGHCVDKFLGDQL</b>	<b>288</b>
<i>E. coli</i>	VESEN-ITERFFVVGKRVSAEVVAAQLVKEVKRYLASTAAVGEYVLADQL	288
<i>H. sapiens</i>	IVFMALANGVSRIKTGPVTLHTQTAIHF AEQIAKAKFIVKKSEDEEDA AK	349
<i>D. melanogaster</i>	IIYMALAVGRSTMRTGKLTNHRTRAINVAEQMTGKFDVAMEPGGQ----	346
<b><i>P. horikoshii</i></b>	<b>IPFLAFSG--GVIWVSEITNHLKTNIWVVE SFLGRIFDVDG-----</b>	<b>327</b>
<i>E. coli</i>	VLPMALAGA-GEFTVAHPSCHLLTNI AVVERFLPVRFSL-----	326
<i>H. sapiens</i>	DTYIIIECQIGMTNPNL	366
<i>D. melanogaster</i>	--MLVSCKGLGHVNKLI	361
<b><i>P. horikoshii</i></b>	<b>---NVGEPGKIRVIRRV</b>	<b>341</b>
<i>E. coli</i>	----IETDGVTRVSIE-	338

**SUPPLEMENTAL FIGURE S4.** Sequence alignment of RtcA proteins. The following species are included in the alignment (including NCBI accession numbers): *Homo sapiens* (O00442), *Drosophila melanogaster* (O77264), *Pyrococcus horikoshii* (O59198), and *Escherichia coli* (AAT48181). The alignment was generated with ClustalW2. Residues interacting with the 3'-p are highlighted in yellow and all other RNA interacting residues are highlighted in blue. Adenosine interacting residues are highlighted in pink. The histidine nucleophile is indicated with an asterisk. *H. sapiens* and *P. horikoshii* RtcA are 39% identical.



**SUPPLEMENTAL FIGURE S5.** RtcA active site depicting the orientation between the RNA 3'-p, adenosine, and the nucleophilic histidine residue. The RNA 3'-p is poised for in-line attack on N<sup>ε</sup> of His307.