Structures of the Noncanonical RNA Ligase RtcB Reveal the Mechanism of Histidine Guanylylation

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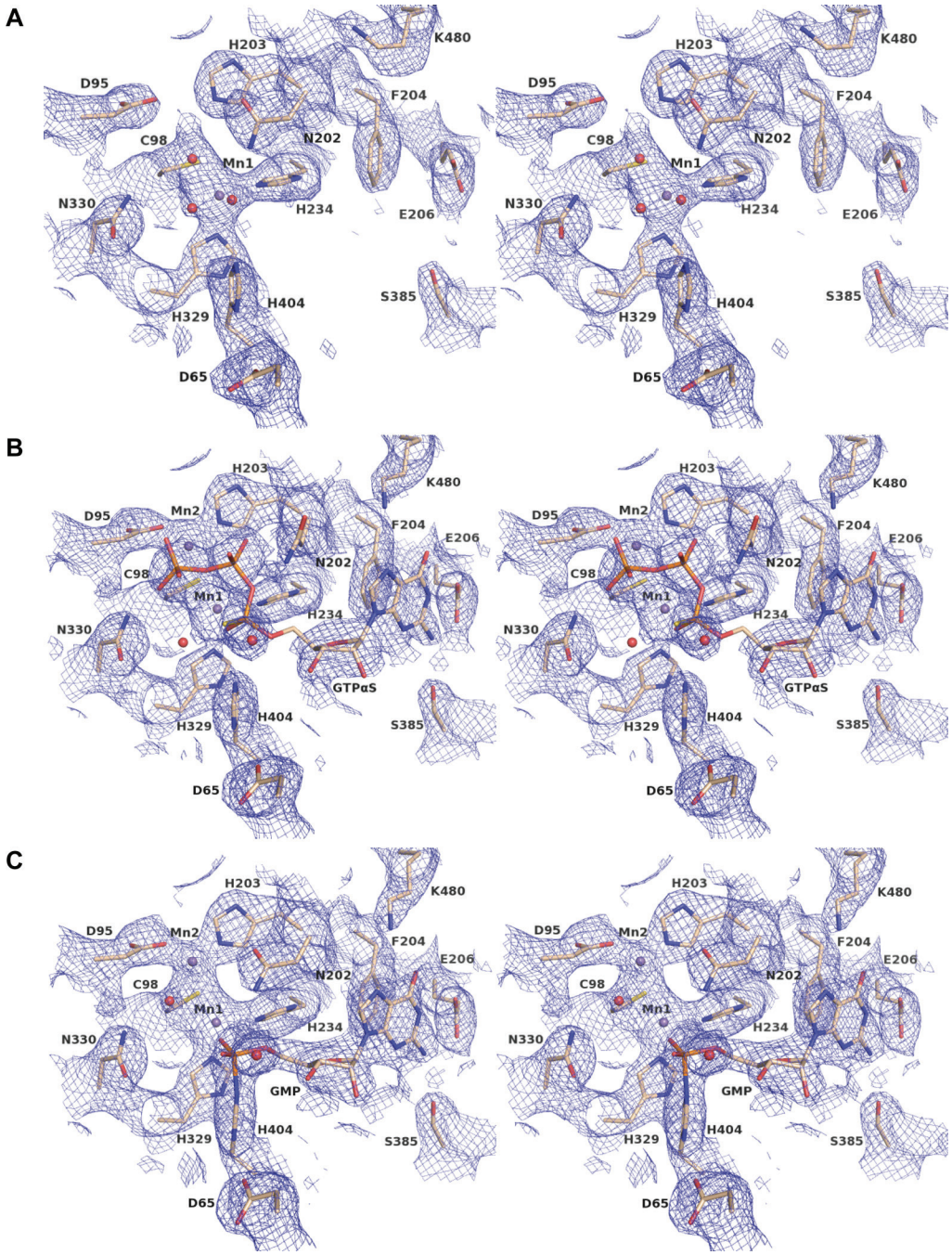

Figure S1. Stereo views (wall-eyed) of the active site of RtcB complexes (subunit A). Blue mesh represents $2F_o - F_c$ density of the refined model contoured at 1.0 σ. (*A*) The RtcB/Mn(II) complex. (*C*) The RtcB/GTPαS/Mn(II) complex. (*D*) The RtcB–GMP/Mn(II) covalent intermediate.

Structure-Guided Mutagenesis of the Guanylate-Binding Pocket. Eight residues of RtcB were found to interact with GMP. Alanine-scanning mutagenesis of these residues revealed their importance for RNA ligation activity. To assay for RNA ligation, two single-stranded 10-nt RNA fragments were used as substrates in ligation reactions. The 5′ RNA fragment had a 3′-P and a 6-carboxyfluorescein (FAM) label at the 5′ terminus. The 3′ RNA fragment had hydroxyl groups at each terminus. Each of the eight RtcB variants tested were inactive in our ligation assays, consistent with a previous report.[1] The essentiality of these residues is also suggested by their strict evolutionary conservation, with the exception of Phe204, which is substituted with tyrosine is some species.[2] These assays also showed that RtcB can use dGTP as cofactor, although ligation activity is reduced substantially.

RNA Ligation Assay. Ligation assays with single-stranded RNA as the substrate used two 10-nt oligonucleotides that were synthesized by Integrated DNA Technologies.[3] The 5′ RNA fragment had a 6-carboxyfluorescein (FAM) label on its 5′ end and was phosphorylated on its 3′ end. The 3′ RNA fragment had hydroxyl groups on each end. The sequence of the 5′ fragment was FAM–5′-AAAUAACAAA-3′–P and the sequence of the 3′ RNA fragment was 5′-AAAUAACAAA-3′. Ligation reactions were performed in 10-μL solutions consisting of 50 mM Bis–Tris buffer, pH 7.0, containing NaCl (300 mM), $MgCl_2$ (0.25 mM), GTP (100 μM), each RNA fragment (1 μM), and RtcB (10 μM). Reaction mixtures were incubated at 70 °C for 1 h prior to the addition of water (40 μL) and RNA-loading buffer (50 μL). Reaction mixtures were subjected to electrophoresis and visualized as described previously.[3]

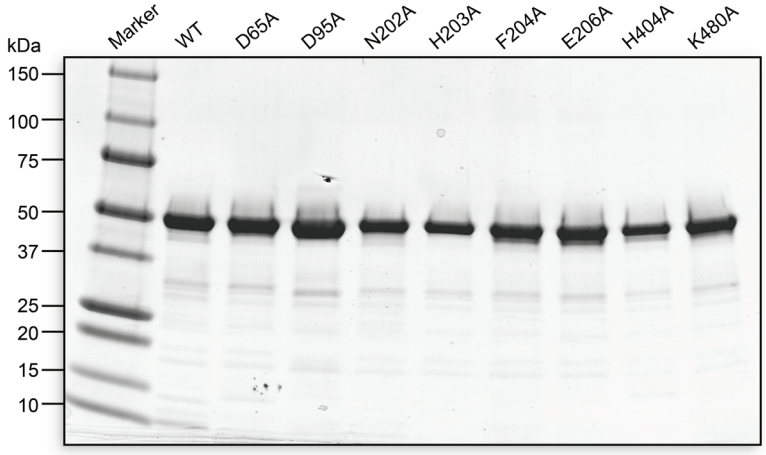

Figure S2. Coomassie-stained SDS–polyacrylamide gel showing purity of RtcB variants. Approximately 4 μg of recombinant RtcB was loaded in each lane. The identities of the variants are indicated at the top and the molecular weight (kDa) is indicated on the left.

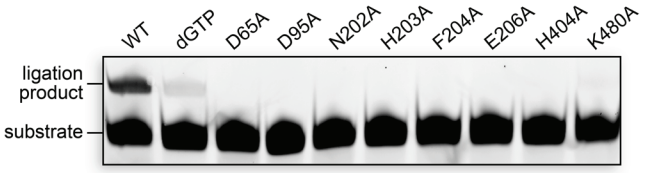

Figure S3. RNA ligation assays using variants of RtcB in which GMP-interacting residues have been replaced with alanine. RtcB is also able to use dGTP as a cofactor, though the ligation efficiency is reduced greatly. The ligase substrates are two 10-nt single-stranded RNAs. The 5′ RNA fragment is labeled with FAM to allow visualization in a urea–polyacrylamide gel; the 3′ RNA fragment has hydroxyl groups at each terminus.

Table S1. Data Collection and Refinement Statistics[a]

	RtcB/Mn(II)	RtcB/GTPαS/Mn(II)	RtcB–GMP/Mn(II)
Data collection			
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions *a*, *b*, *c* (Å)	81.04, 137.70, 149.01	81.11, 138.91, 149.47	81.11, 138.91, 149.47
Radiation Source	21-ID-F APS	21-ID-G APS	21-ID-G APS
Wavelength	0.97872Å	0.97857Å	0.97857Å
Resolution (Å)	49.49–2.34 Å (2.43–2.34)	49.75–2.30 Å (2.39–2.30)	49.75–2.4 Å (2.48–2.40)
R_{sym} or R_{merge}	0.110(0.832)	0.148(0.842)	0.139(0.990)
Mean I / sI	10.34(2.22)	9.13(1.40)	11.37(2.15)
Completeness (%)	99.16(93.41)	97.44(78.50)	99.25(92.40)
Redundancy	7.1(6.8)	7.1(4.7)	7.2(6.8)
Refinement			
Resolution (Å)	49.49–2.34 Å (2.43–2.34)	49.75–2.30 Å (2.39–2.30)	49.75–2.4 Å (2.48–2.40)
Unique reflections	70143(6530)	73441(5841)	66436(6089)
R_{work} / R_{free}	0.156/0.194(0.192/0.249)	0.165/0.205(0.257/0.286)	0.152/0.192(0.211/0.267)
B-factors (Å^2)			
Protein	18.2	32.3	34.8
Solvent	24.5	37.4	40.6
Ramachandran plot	96% favored	96% favored	97% favored
R.M.S. deviations			
Bond lengths (Å)	0.013	0.017	0.015
Bond angles (°)	1.510	1.622	1.482
Protomers	2	2	2
Protein residues	960	960	960
Heteroatoms	2 Mn(II) ions	4 Mn(II) ions	4 Mn(II) ions
	13 sulfates	2 GTPαS	1 GMP
	2 sucrose	19 sulfates	19 sulfates
	416 waters	2 sucrose	2 sucrose
		398 waters	551 waters
PDB ID	4isj	4isz	4it0

[a] Values in parentheses are for highest-resolution shell.

References

1. Englert, M. et al. (2012) Structural and mechanistic insights into guanylylation of RNA-splicing ligase RtcB joining RNA between 3′-terminal phosphate and 5′-OH, *Proc. Natl. Acad. Sci. U.S.A. 109*, 15235–15240.
2. Popow, J. et al. (2010) HSPC117 is the essential subunit of a human tRNA splicing ligase complex, *Science 331*, 760–764.
3. Desai, K.K., and Raines, R.T. (2012) tRNA ligase catalyzes the GTP-dependent ligation of RNA with 3′-phosphate and 5′-hydroxyl termini, *Biochemistry 51*, 1333–1335.