Supplemental Material

Structural basis for the GTP specificity of the RNA kinase domain of fungal tRNA ligase

Barbara S. Remus, Yehuda Goldgur, and Stewart Shuman

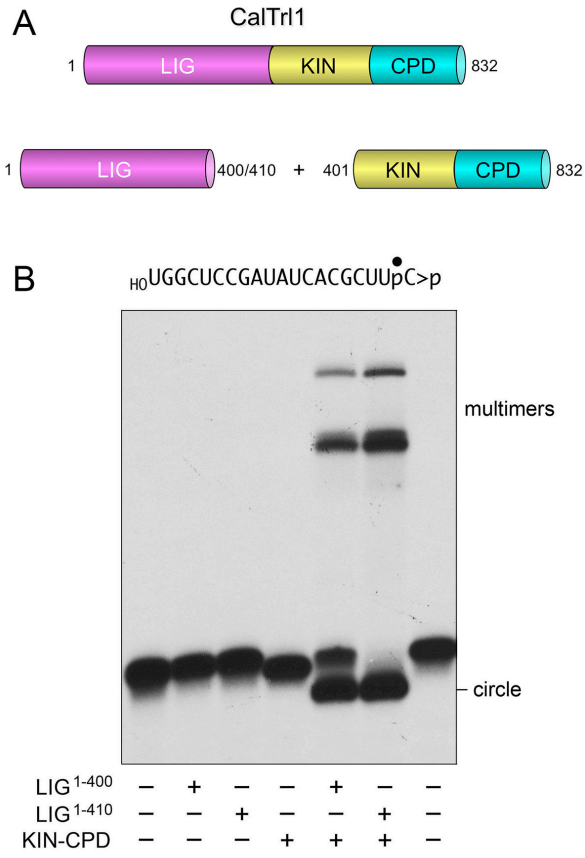

Figure S1. ***Candida albicans* Trl1**. (A) CalTrl1 consists of N-terminal ligase (LIG), central kinase (KIN), and C-terminal cyclic phosphodiesterase (CPD) modules. The indicated LIG and KIN-CPD fragments were produced as separate recombinant proteins. (B) Reconstitution of RNA end-joining with isolated LIG and KIN-CPD domains. Reaction mixtures (10 μl) containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 2 mM DTT, 10 mM $MgCl_2$, 100 μM GTP, 20 nM ^{32}P-labeled 20-mer $_{HO}$RNA>p substrate (depicted at top, with the ^{32}P-label denoted by ●), and 100 nM LIG or KIN-CPD domains (where indicated by +) were incubated at 22°C for 30 min. The reactions were quenched with an equal volume of 90% formamide, 30 mM EDTA. The labeled RNAs were resolved by urea-PAGE. An autoradiogram of the gel is shown. The positions of the ligated circle and multimer products are indicated on the *right*.

Table S1

Crystallographic data and refinement statistics

	Native Cal KIN	Hg derivative
Data collection		
Beamline	APS 24-ID-C	APS 24-ID-C
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å) α, β, γ (°)	 49.9, 56.3, 82.6 90, 90, 90	 51.76, 51.15, 88.96 90, 90, 90
Resolution (Å)	50–2.2 (2.25–2.2)	50–2.3 (2.35–2.3)
Wavelength (Å)	0.9791	0.9791
R_{pim}	0.081 (0.336)	0.055 (0.212)
CC(1/2)	0.984 (0.715)	0.991 (0.916)
$\langle I \rangle / \langle \sigma I \rangle$	11.0 (1.5)	15.1 (2.3)
Completeness (%)	90.4 (70.1)	99.0 (96.4)
Redundancy	2.2 (1.4)	3.9 (2.8)
Unique reflections	12608	11700
Phasing		
Number of Hg sites		5
Figure of merit		0.655
Refinement		
R_{work} / R_{free}	0.187 / 0.252	
B-factors (Å^2) Average / Wilson	40.8 / 30.5	
RMS deviations bond lengths (Å) bond angles (°)	 0.007 0.949	
Ramachandran plot % favored % allowed outliers	 97.6 2.4 0	
Model contents		
Protomers / ASU	1	
Protein residues	213	
Mg ion	1	
Ligand	GDP	
Water	111	
PDB ID	5U32	

Values in parentheses refer to the highest resolution shell.

R_{free} set consists of 10% of data chosen randomly against which structures were not refined.

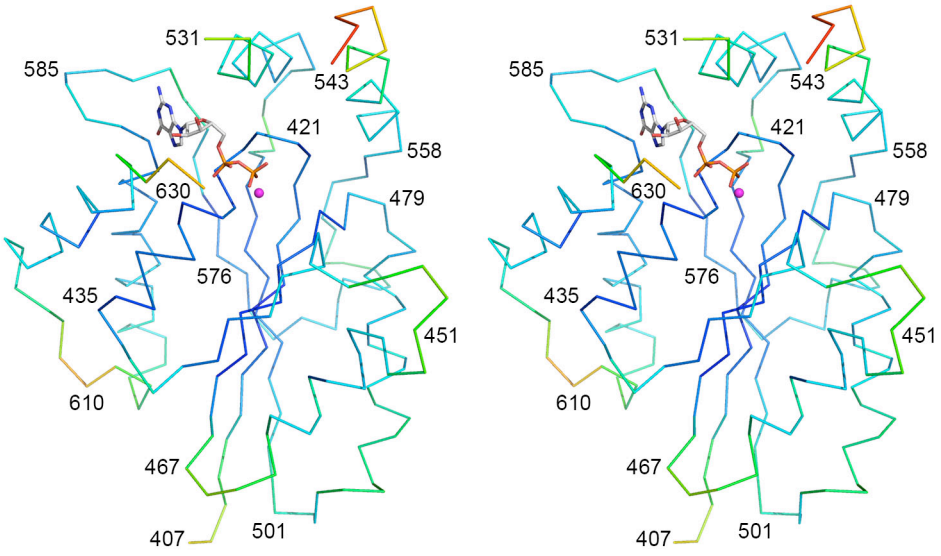

Figure S2. Stereo view of a main-chain trace of the kinase tertiary structure, colored according to the B-factors of the Cα atoms, from the lowest values in dark blue (Val416/Thr427/Ala508 = 23.7/25.9/25.8) to the highest in red-orange (Leu543/Asp544/Glu545 = 86.3/85.3/86.5). GDP and Mg^{2+} are depicted as a stick model and magenta sphere, respectively.

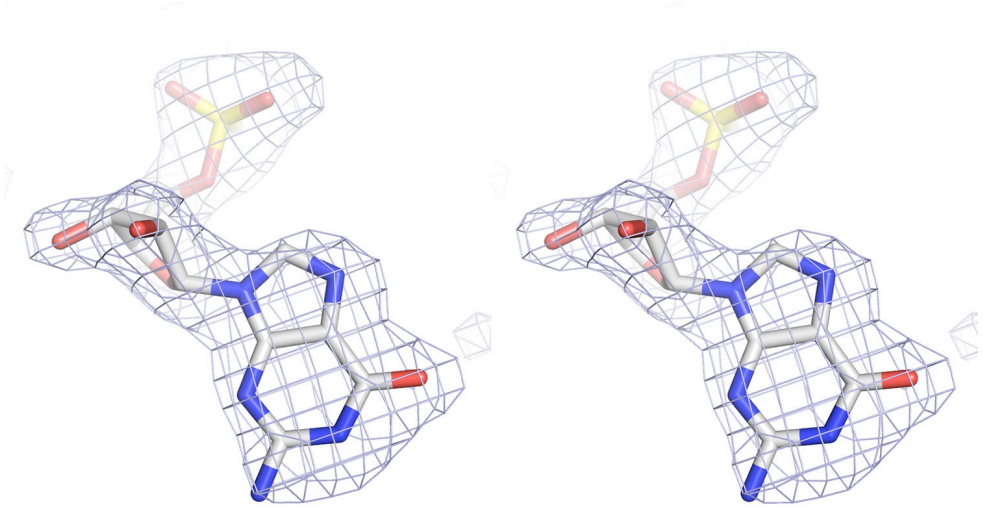

Figure S3. **Electron density for a guanosine nucleotide in the kinase active site**. Shown is a stereo view of a simulated annealing omit map (Fo-Fc) of the electron density for the GMP moiety of GDP bound to the *Candida* Trl1 kinase domain, colored in light blue mesh and contoured at 3 σ. The map was calculated after GDP, waters, and magnesium ion were omitted and the model was subject to torsion angle simulated annealing from 5000 K. The nucleotide is shown as a stick model in an orientation that highlights the electron density for the guanine nucleobase. (The GDP beta phosphate density and model are slabbed out for simplicity.)

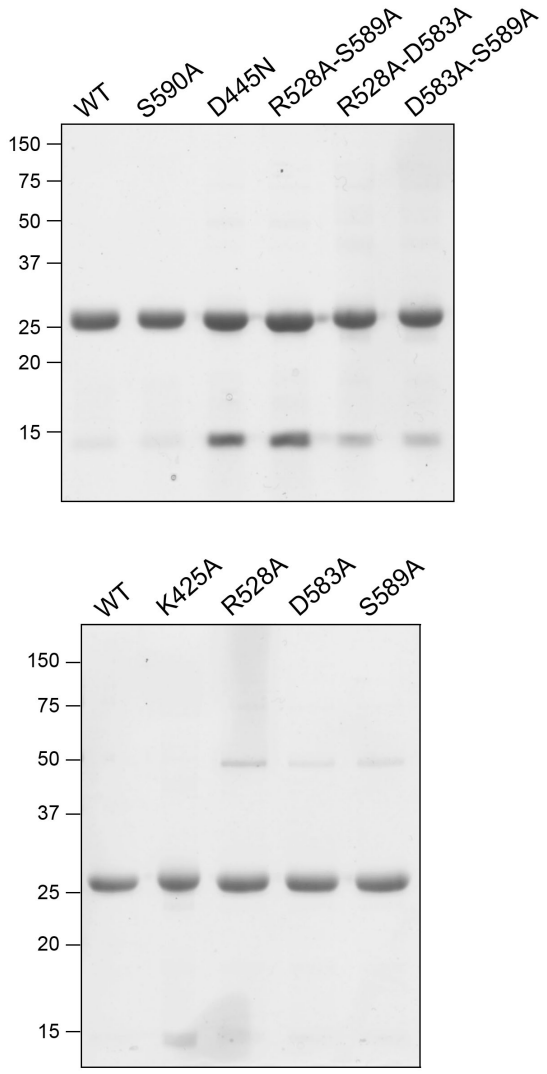

Figure S4. ***Candida* Trl1 KIN mutants**. Aliquots (4 µg) of the recombinant wild-type KIN domain (WT) and the indicated mutants were analyzed by SDS-PAGE. The Coomassie blue-stained gel is shown. The positions and sizes (kDa) of marker polypeptides are indicated on the *left*.