COCRYSTAL STRUCTURE DETERMINATION OF A PSEUDOURIDINE SYNTHASE, A NUCLEOTIDE-FLIPPING RNA-MODIFYING ENZYME, AT ALS BEAMLINE 5.0.2

C. Hoang and A.R. Ferré-D'Amaré
Division of Basic Sciences, Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue North, Seattle, WA 98109-1024, USA

SUMMARY

Pseudouridine (Ψ) synthases catalyze the isomerization of specific uridines in cellular RNAs to pseudouridines and may function as RNA chaperones. TruB is responsible for the Ψ residue present in the T loops of virtually all tRNAs in the cell. The close eukaryotic homolog Cbf5/dyskerin is the catalytic subunit of box H/ACA snoRNPs that catalyze modification of ribosomal RNA. The 1.85 Å resolution structure of TruB bound to an inhibitor RNA [1] shows that this enzyme recognizes the preformed three-dimensional structure of the T loop. It gains access to its substrate uridyl residue by flipping out the nucleotide, and thus disrupts tertiary, or long-range, interactions between the T and D loops of tRNA. This is the first structure determination of an RNA base-modifying enzyme bound to a substrate.

BACKGROUND

Pseudouridine (Ψ) is the most abundant modified nucleotide in RNA. The isomerization of U into Ψ involves breakage of the glycosidic bond connecting the ribose to the pyrimidine base, rotation of the detached base, and reconnection through C5 (Fig.1). These enzymes require no cofactors or external sources of energy.

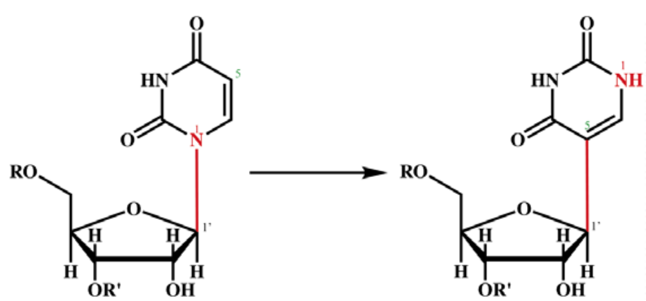

Figure 1. Isomerization of uridine (left) into Ψ catalyzed by Ψ synthases. Note how the glycosidic bond (in red) in uridine is replaced by a carbon/carbon bond in Ψ. R and R' denote the RNA chain to either side of the site of modification.

EXPERIMENTAL DESIGN

In order to understand how a Ψ synthase recognizes its target RNA, and how the post-transcriptional catalytic transformation is achieved, we determined the structure of *Escherichia coli* TruB in complex with an RNA-based inhibitor (Fig. 2A). RNAs with a 5-fluorouracil (5FU) residue at the site of modification are tight mechanism-based inhibitors of tRNA Ψ synthases [2]. Since the regioselectivity and kinetics of pseudouridylation are the same on full-length tRNAs and RNAs comprised of the isolated T stem and loop (TSL RNAs), all determinants of specific Ψ55 synthase-tRNA recognition must lie within this segment of tRNAs [3]. A 22-nucleotide (nt) TSL with 5FU at the site of modification produced well-ordered cocrystals. The TruB cocrystal structure reveals that this Ψ synthase gains access to its substrate by flipping out nucleotide 55 of tRNA. In the TruB-TSL complex, the bases of nucleotides 55, 56, and 57 are everted from the position they would assume within the helical stack of isolated, folded tRNA (Fig. 2B). In folded tRNAs, these three nucleotides in the T loop contact the D loop to stabilize the tertiary structure of the RNA.

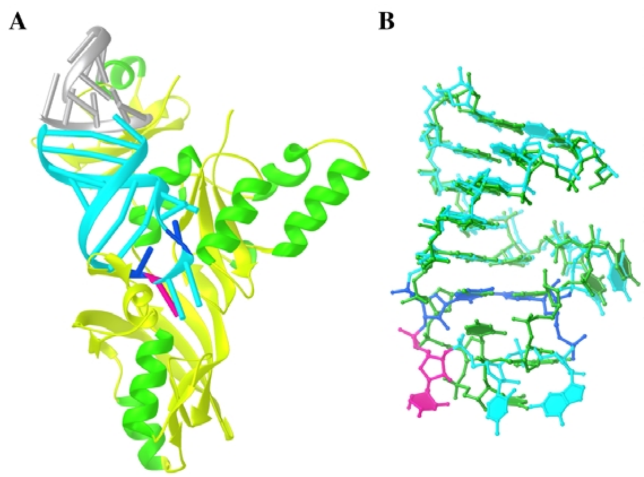

Figure 2. Three-dimensional structure of the Ψ synthase TruB complexed to a T stem-loop RNA. **A**.Ribbon representation of the complex. Protein helices are dark green, strands and loops are light green. The TSL RNA is light blue, except for nucleotides U54 and A58 that make a conserved reverse Hoogsteen pair colored dark blue, and U55 (the site of pseudouridylation) in purple. A segment of a symmetry-related RNA that extends the A-form helix is shown in light gray. **B.** Superposition of the TSL bound to TruB (colored as in Figure 1) with the corresponding residues from the structure of intact, folded $tRNA^{Phe}$ (in green). Three nucleotides at the apex of the T loop are flipped out of the helical stack by binding to TruB.

STUDIES CONDUCTED AT THE ADVANCED LIGHT SOURCE

Our TruB cocrystals have the symmetry of space group *C*2, and unit cell dimensions a = 145.05 Å, b = 40.36 Å, c = 77.99 Å and β = 110.60°. Experimental phases were calculated at 2.0 Å resolution using MAD data measured at beamline 5.0.2 using a cocrystal containing a selenomethionine version of TruB. The MAD diffraction data were collected at three wavelengths corresponding to the peak, inflection point, and high energy remote for selenium as determined from a flurorescence energy scan of the actual crystal. Given the low symmetry of the crystal, in order to obtain complete anomalous data sets, 360° of data were collected at the both the peak and remote wavelengths. A 180° of data were collected at the inflection point. The three X-ray energies remained very stable over the eleven hours of data collection. 'Solvent flattening' and phase extension to 1.85 Å resolution produced an experimental electron density map into which most of the protein and RNA residues could be built unambiguously (Fig. 3). The structure has been refined to an R factor of 18.4% and a free R factor of 21.2%.

The cocrystal structure of TruB reveals for the first time how a Ψ synthase recognizes its substrate and suggests how it may function in promoting RNA folding [1]. This high resolution structure is the starting point for unravelling the mechanism of action of these phylogenetically conserved enzymes, and also for exploring their possible roles in maturation and assembly of RNAs.

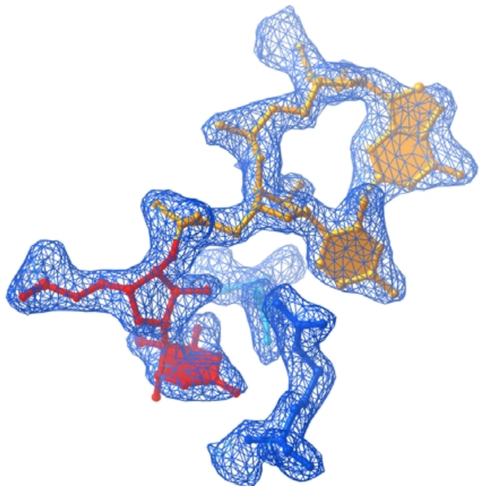

Figure 3. Portion of the 1.85 Å resolution 'solvent flattened' MAD experimental electron density map corresponding to part of the active site of TruB, contoured at 1.5 standard deviations above mean peak height.

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Principal Investigator: Adrian R. Ferré-D'Amaré, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109-1024. Email: aferre@fhcrc.org. Telephone: 206-667-3622.