The extent of B-form structure in a 6S RNA variant that mimics DNA to bind to the *Escherichia coli* RNA Polymerase

Nilesh K. Banavali^{1,2,*}

1: Laboratory of Computational and Structural Biology

Division of Genetics, Wadsworth Center, NYS Department of Health

2: Department of Biomedical Sciences, School of Public Health

State University of New York at Albany

email: nilesh.banavali@health.ny.gov

Biggs Laboratory, Wadsworth Center, NYS Department of Health, Empire State Plaza,

PO Box 509 Albany, NY 12201-0509

Tel: 518-474-0569

Fax: 518-474-3181

Website: <http://www.wadsworth.org/resnres/bios/banavali.htm>

Abstract

In a recent article by Darst and coworkers, it was found that a non-coding 6S RNA variant regulates a bacterial RNA polymerase by mimicking B-Form DNA, and a few different nucleic acid duplex parameters were analyzed to understand the extent of B-form RNA structure. In this manuscript, a different structural analysis based on conformational distance from canonical A-form and B-form single-strand structures is presented. This analysis addresses the occurrence and extent of both local and global B-form structure in the published 6S RNA variant model.

In a recent article describing the structure of non-coding 6S RNA variant bound to an RNA polymerase holoenzyme, Darst and coworkers describe an overall RNA architecture that partially mimics B-form DNA.¹ In their 3.8 Å single particle cryo-EM structure, they show an average major groove width resembling the B-form, and an average minor groove width resembling the A-form, in the duplex regions of the 6S RNA variant (termed 6S RNA* henceforth) that are long enough to measure groove widths. They also show the pitch and rise parameter values to be nearer those for the B-form, while the sugar pucker, phosphate-phosphate distances, and twist parameter values to be nearer those for the A-form. This analysis of nucleic acid duplex parameters shows that the 6S RNA structure has features of both A-form and B-form architectures.

Proximity to A-form and B-form reference structures can also be assessed using a Root Mean Square Deviation (RMSD) measure,² and we have performed such analysis on known RNA crystal structure conformations of 3 Å or greater resolution at the individual single strand dinucleotide (SSD) level.³ This analysis does not require duplex structures, and can assess relative proximity to A- and B-form structures at a local or global level. We showed that RNA SSDs approach a B-form structure in many instances,³ and this local B-form structure seems to be achieved through both C2'-endo sugar conformations or base stacking patterns that resemble B-form DNA stacking.⁴ Interestingly, the base RMSD values show a greater propensity for B-form structures than the sugar-phosphate backbone at the SSD level, which suggests an under-representation of B-form-like sugar-phosphate conformations for RNA in the PDB models. While duplex RNA usually adopts an A-form structure with its furanose sugars assuming C3'-endo pucker conformations,⁵ the C2'-endo pucker is present in some functional RNA motifs, and may possibly be implicated in RNA folding.^{6,7}

It is possible to apply such RMSD analysis to understand the extent of local and global B-form conformations in the 6S RNA* structure (PDB ID: 5VT0, Chain R). Figure 1A shows the scatter plot of each 6S RNA* SSD conformation in 2D RMSD space for all, base, and backbone non-hydrogen atoms, with the RMSD values computed with respect to canonical A- and B-form SSD reference structures. These RMSD values are normalized by the RMSD difference between the A- and B-form SSD structures, thus setting the RMSD between the A- and B-forms to a value of 1.0, irrespective of the SSD sequence. The interpolation line between the A- and B-forms is shown as a dotted blue line. The dividing solid red line separates

the conformational space into two regions, one nearer to the B-form (bordered by a dotted cyan line), and another closer to the A-form (bordered by a dotted pink line). If all SSDs are simply separated into ones relatively closer to the A-form or the B-form, then a majority of the SSDs are clustered near the canonical A-form, but some SSDs clearly cross over to the B-form, mostly in conformational regions further away from the canonical B-form. This behavior was also observed in our larger crystallographic survey,³ such that crossing over to the B-form region seems to be easier for RNA if it moves away from both canonical forms, and the region very close to the canonical B-form structure does not seem to be accessible.

To see which SSDs of the 6S RNA* structure might assume a B-form conformation reasonably close to the canonical B-form, a normalized RMSD cutoff was used to limit the distance from the interpolation line (1.0 in this case, shown as a green dotted line). The conformations with base non-hydrogen atom RMSD values that fall in the B-form region within this cutoff distance are shown in red in Figure 1B. This analysis shows that, even for the base moieties more likely to show B-form conformations, there are only six B-form SSD conformations distributed throughout the entire RNA structure that are nearer the canonical B-form. To assess the A-form or B-form proximity for longer stretches of the RNA strands, the single stranded segments that had continuous stacking were identified. These stretches consisted of residues 32-42, 43-47, 58-68, 72-81, 83-85, 99-107, 112-134, 137-142, and 144-153. The RMSD values for different components of these single stranded regions are listed in Table 1. The stacked single strand segments showing greater proximity to the B-form than the A-form at these longer length scales (for the base non-hydrogen atoms) are shown in red in Figure 1C. It can be seen that a substantial part of the 6S RNA variant structure assumes overall single stranded conformations that resemble the B-form more than the A-form.

The proportion of the 6S RNA* SSDs merely in the B-form half of the 2D RMSD space is less than 35% of the structure, while that closer to the canonical B-form is about 5% of the structure. In contrast, the proportion of SSDs merely in the A-form half (68%) is similar to the proportion close to the canonical A-form (60%). This suggests that the 6S RNA* can mimic DNA by having only a third of its SSDs resembling the B-form more than the A-form, and only a twentieth of its SSDs being nearer the canonical B-form structure. The propensity of B-form structure in RNA crystal structure SSD conformations that we assessed previously using the base non-hydrogen RMSD is also near 5%.³ This 6S RNA* structure therefore suggests that RNA

may be able to mimic DNA functionally by crossing over into the B-form 2D RMSD space, even through just a substantial minority of its SSDs assuming conformations closer to the B-form than the A-form.

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Table 1: Canonical form proximity of stacked single strand segments of the 6S RNA variant structure.

Residues	D_{RMSD} All	D_{RMSD} Base	D_{RMSD} Backbone	$RMSD_A$ All	$RMSD_B$ All	$RMSD_A$ Base	$RMSD_B$ Base	$RMSD_A$ Backbone	$RMSD_B$ Backbone
32-42	-0.28	0.12	-0.40	0.50	0.78	0.70	0.59	0.42	0.82
43-47	-0.60	-0.61	-0.60	0.45	1.05	0.60	1.22	0.43	1.03
58-68	-0.15	0.10	-0.23	0.74	0.89	0.89	0.79	0.69	0.92
72-81	-0.34	-0.21	-0.39	0.51	0.85	0.73	0.95	0.44	0.83
83-85	-0.41	-0.14	-0.46	1.19	1.60	2.00	2.15	1.08	1.54
99-107	-0.48	-0.31	-0.52	0.45	0.93	0.56	0.87	0.42	0.94
112-134	-0.14	0.09	-0.25	0.54	0.68	0.64	0.55	0.49	0.74
137-142	-0.34	0.07	-0.42	0.64	0.98	1.27	1.20	0.53	0.95
144-153	-0.27	0.04	-0.35	0.55	0.83	0.70	0.67	0.51	0.86

$D_{RMSD} = RMSD_A - RMSD_B$, where $RMSD_A$ and $RMSD_B$ are RMSDs of a particular structure from the canonical A- and B-forms, respectively. Structures in the A-form half of 2D RMSD space have negative D_{RMSD} values, and structures in the B-form half have positive D_{RMSD} values. All RMSD values are calculated after a Kabsch least squared fit⁸ of the two structures using either all non-hydrogen atoms (All), or using only the non-hydrogen atoms of its base moieties (Base), or using only the non-hydrogen atoms of its sugar-phosphate backbone (Backbone). All RMSD values are normalized by the corresponding difference in RMSD between the canonical A- and B-form reference structures.

Figure 1: Analysis of SSD and longer RNA single strand B-form conformations in the 6S RNA variant structure bound to the *E. coli* RNA polymerase. (A) SSD conformations scatter plots for all SSD non-hydrogen atoms (top), base SSD non-hydrogen atoms (center), and sugar-phosphate backbone SSD non-hydrogen atoms (bottom) in normalized 2D RMSD space from the canonical A-form and B-form structures. The interpolation line between the two canonical forms is in dotted blue, the dividing line between A- and B-form regions is in solid red, the B-form region border is in dotted cyan, and the A-form region border is in dotted pink. (B) Structural location of SSDs (red) near the canonical B-form structure, as judged by presence in the B-form half and an additional cutoff of 1.0 in the perpendicular distance from the dotted blue interpolation line. (C) Indication of overall B-form segments (red) of extended stacked single strand regions of the 6S RNA structure according to base non-hydrogen atom RMSD values.