Table 2. Summary of binding affinities and relative binding affinity of Tex and Tex mutants based on gel mobility shift analysis

Protein	K_d[a] (μM)	Percentage of binding relative to WT
25 nt ssRNA		
WT Tex	0.21±0.05	100
ΔS1 Tex	nd	<0.1
R718E	28.4±11.4	0.74±0.30
F668D/F671D	13.7±0.7	1.53±0.08
F668D	15.5±5.5	1.35±0.48
F671D	9.8±1.7	2.14±0.37
H683E	10.7±3.5	1.96±0.64
25 bp dsRNA		
WT Tex	4.2±1.47	100
ΔS1 Tex	nd	<0.1
R718E	46.9±6.3	8.96±1.20
F668D/F671D	21.2±11.5	19.8±10.6
25 bp dsDNA		
WT Tex	3.7±0.15	100
ΔS1 Tex	nd	<0.1
R718E	48.3±5.2	7.66±0.82
F668D/F671D	20.4±3.8	18.1±3.38
25 nt ssDNA		
WT Tex	5.1±1.5	100
ΔS1 Tex	nd	<0.1
R718E	118.7±10.7	4.30±0.39
F668D/F671D	55.3±6.0	9.22±1.00

nd, no binding detected.
[a] Values represent the average K_d from multiple experiments ($2 \le n \le 9$)±standard error.

accession code: 1YZ6, 79 C^{α}, RMSD = 1.8 Å), and the archeal homolog of the RNAPII subunit RPB7 (PDB accession code: 1GO3, 72 C^{α}, RMSD = 1.8 Å) (Fig. 5). These proteins are all reported to bind RNA transcripts in a sequence-nonspecific manner with the interaction being important for RNA decay,[34] general translation initiation,[36] and transcription initiation.[37,38] Given the close alignment of these conserved residues, it is likely that cellular substrates for Tex are also sequence-nonspecific RNA transcripts.

Although putative RNA-binding residues align well among some S1 proteins, precise alignment of critical residues does not appear to be required for binding similar RNA substrates. The crystal structures of the ribonucleases RNase E (PDB accession code: 2C0B) and RNase II (PDB accession code: 2IX1) represent costructures of RNA-bound S1 domains that interact with RNA independent of sequence. S1 residues making significant contacts in these structures do not align precisely with one another even though the general composition of side chains is maintained and the interaction occurs across the same S1/OB binding cleft. When aligning Tex S1 with these structures, a similar theme is observed, where F668, F671, H683, and R718 are clustered in the same binding cleft as equivalent RNase E and RNase II residues. Based on these observations, we propose a model for Tex binding to ssRNA via the S1/OB binding cleft (Fig. 5b).

In order to map the nucleic acid binding surface further and to test the binding model, we assayed a variety of Tex S1 domain point mutants for RNA binding. Mutation of the conserved S1 binding cleft residues F668, F671, H683, and R718 to aspartate or glutamate disrupts ssRNA binding by at least 46-fold [<2.2% binding relative to wild-type (WT) Tex] (Table 2), with a 135±50-fold reduction (0.74±0.30% of WT Tex) in ssRNA binding when the mutant R718E was assayed by gel shift. The double mutation F668D/F671D results in a 65±3-fold reduction (1.53±0.08% of WT Tex) in ssRNA binding and implies that hydrophobic base-stacking and/or packing interactions on the S1 surface are additionally important. Consistent with the model that all nucleic acid binding in our assay conditions is to the S1 domain and that ssRNA is the preferred ligand, affinities for all substrates were reduced in the S1 point mutants, with the greatest effect seen on binding of ssRNA.

Overall, the structural and binding data indicate that the S1 domain is a highly dynamic module that is required for nucleic acid binding and displays a strong preference for ssRNA. Binding is likely to be sequence independent, as has been found for the majority of other described S1-domain-containing proteins, including those that align well with Tex. Although S1 is the primary nucleic acid binding domain, we cannot rule out minor contributions to binding from other regions, and it is possible that specific *in vivo* contexts, such as binding to another partner, might open additional surfaces for binding to nucleic acid substrates.

Consideration of minimal oligonucleotide length required for Tex binding suggests that S1 is not the only region that binds the various nucleic acid substrates. Ten-nucleotide oligonucleotides do not bind Tex in our assay, and a significant decrease in binding affinity was observed for ssRNA lengths less than 20 nt. An ssRNA molecule of 10 nt or longer would extend beyond the available binding surface of the S1 domain, implying that additional contacts outside the S1 domain occur in our assay. This is a familiar theme for S1-containing proteins. For example, the RNase E S1 domain is a dynamic module that serves as a molecular clamp for correctly orienting ssRNA substrate.[35] Given the modularity of the Tex S1 domain, the presence of multiple other nucleic acid binding domains, and a minimal substrate length that spans a surface larger than that offered by the S1 domain, one attractive possibility is that Tex may utilize an S1 molecular clamp binding model similar to RNase E.[35]

Putative nuclease activity

Based on the presence of the RNase H fold YqgF domain and the observation that Tex negatively regulates transcription when overexpressed, Tex is predicted to have ribonuclease activity.[1,2] In conflict with this prediction, however, we have not detected nuclease activity. There is no indication of nucleic acid degradation in our gel shift experiments. We performed a qualitative nuclease assay in which the positive controls (RNase T1 and micrococcal nuclease) were active, but activity was at background levels for Tex (data not shown). In addition, we