observed no differences in nucleic acid binding or the very low background nuclease activity between WT Tex and a double mutant in which two putative catalytic residues in the YqgF domain (Asp335 and Glu421) were changed to alanine (data not shown). The lack of nuclease activity could be explained by the fact that the Tex YqgF domain lacks a critical and highly conserved carboxylate residue that is required for metal coordination in known RNase H fold ribonucleases (e.g., D141 of *E. coli* RuvC) (Fig. 8). Therefore, in contrast to the earlier prediction,[1,2] these data suggest that Tex is not a ribonuclease.

Implications for Spt6

Many S1-domain-containing proteins are factors involved in general processes such as transcription, translation initiation, and mRNA decay.[28,31,36,37] Consistent with this idea, comparative genomic and evolutionary studies suggested that Tex represents a bacterial ortholog of the eukaryotic transcription elongation factor Spt6.[5,7] Although Spt6 is twice the size of Tex, it is predicted to possess YqgF, HhH, and S1 domains in the same order and to possess 15% sequence identity (27% similarity) over these regions (Fig. 9a). The predicted secondary structural elements of the Spt6 sequence also show good agreement with the Tex structure (data not shown).

The nature of the structural similarity between Tex and Spt6 is further clarified by aligning the Spt6 sequence with the Tex structure. In particular, all of the differences in sequence length between Tex and Spt6 (e.g., Spt6 insertion sequence) occur on the surface of the Tex structure, which appears able to accommodate additional sequences without disrupting the core structural scaffold (data not shown). Additionally, evolutionary conservation scores based on Tex- and Spt6-related sequences were assigned for each Tex amino acid residue and mapped onto the Tex structure using the ConSurf server.[19] Most of the conserved residues identified by this method appear to be involved in packing interactions in the Tex structure. These observations suggest that Spt6 retains the core Tex structure, with variations on the periphery, along with additional domain features at the N- and C-terminal ends (described below).

Unlike Tex, Spt6 possesses an SH2-like domain C-terminal to the S1 domain.[39] Given the proximity of the SH2-like sequence to the S1 domain, it is likely that this domain lies along one face of the Spt6 structure, as indicated in Fig. 9. The Spt6 SH2-like domain is reported to mediate interactions with the C-terminal domain of RNAPII.[12] Based on the mobility of the S1 domain observed in the Tex structures, it is likely that Spt6 binds RNAPII via a flexible tether.

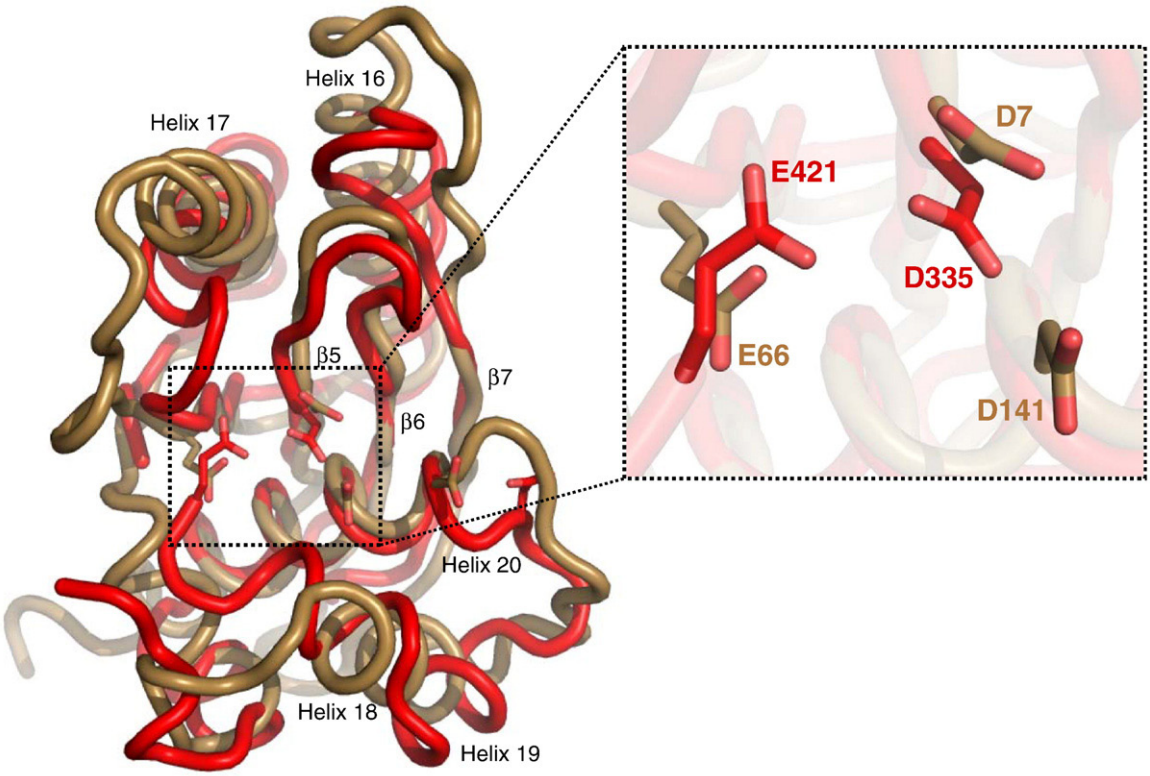

Fig. 8. The Tex Yqgf (red) superimposed on the *E. coli* RuvC (sand) (PDB accession code: 1HJR, 120 C^{α}, RMSD = 3.0) and an exploded view of the catalytic center. The RuvC catalytic residues D7, E66, D141, and D138 are shown in stick representation aligned with Tex residues D335, D421, and D441 that share the same basic geometric orientation in the catalytic center. Although three of the four conserved catalytic residues are present in Tex, there is no equivalent acidic residue present at the location corresponding to RuvC D141.