doi:10.1016/j.jmb.2008.01.096 J. Mol. Biol. (2008) 377, 1460–1473

JMB

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Crystal Structure and RNA Binding of the Tex Protein from *Pseudomonas aeruginosa*

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Received 18 December 2007; received in revised form 23 January 2008; accepted 25 January 2008
Available online 12 February 2008

Edited by J. Doudna

Tex is a highly conserved bacterial protein that likely functions in a variety of transcriptional processes. Here, we describe two crystal structures of the 86-kDa Tex protein from *Pseudomonas aeruginosa* at 2.3 and 2.5 Å resolution, respectively. These structures reveal a relatively flat and elongated protein, with several potential nucleic acid binding motifs clustered at one end, including an S1 domain near the C-terminus that displays considerable structural flexibility. Tex binds nucleic acids, with a preference for single-stranded RNA, and the Tex S1 domain is required for this binding activity. Point mutants further demonstrate that the primary nucleic acid binding site corresponds to a surface of the S1 domain. Sequence alignment and modeling indicate that the eukaryotic Spt6 transcription factor adopts a similar core structure. Structural analysis further suggests that the RNA polymerase and nucleosome interacting regions of Spt6 flank opposite sides of the Tex-like scaffold. Therefore, the Tex structure may represent a conserved scaffold that binds single-stranded RNA to regulate transcription in both eukaryotic and prokaryotic organisms.

Keywords: transcription; S1 domain; Tex; x-ray crystallography; Spt6

Introduction

The Tex (*t*oxin *ex*pression) protein was originally described in *Bordetella pertussis* as an essential protein involved in expression of critical toxin genes.[1] Tex is a relatively large protein with a domain architecture consisting of several nucleic acid binding domains predicted from primary sequence. The presence of these domains supports the proposal that Tex is a transcription factor that functions in toxin expression and/or pathogen fitness.[1–3] Tex displays a remarkably high degree of identity and similarity across a host of significant pathogens. For example, Tex from *Pseudomonas aeruginosa* shares 65% identity and 78% similarity (at the amino acid level) with Tex from *Vibrio cholerae* (the causative agent of cholera). Similar degrees of identity are seen with Tex proteins from *Shigella flexneri* (the causative agent of dysentery) and *Yersinia pestis* (the causative agent of plague).

Despite being ubiquitous and extremely well conserved, the molecular functions of Tex remain enigmatic. Insight into Tex function is derived from several bacterial studies. Aside from its role in expression of toxin gene products in *B. pertussis*, the *tex* gene from *P. aeruginosa* (PA5201) appears to play an important role in pathogenesis, being required for lung infection in a chronic disease model.[4] In *Streptococcus pneumoniae*, Tex does not alter expres-

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Abbreviations used: RNAP, RNA polymerase; HtH, helix–turn–helix; ssRNA, single-stranded RNA; Se-Met, selenomethionine; PDB, Protein Data Bank; HhH, helix–hairpin–helix; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; OB, oligonucleotide/oligosaccharide binding; dsRNA, double-stranded RNA; FP, fluorescence polarization; WT, wild-type; BME, β-mercaptoethanol; NSLS, National Synchrotron Light Source.

0022-2836/$ - see front matter. Published by Elsevier Ltd.