in the free DNA while the latter is essentially straight, thus requiring a higher deformation energy. However there may be more to the story. For example the two dodecamers form complexes with the BPV-1 E2 protein which are structurally quite similar to those formed with HPV-18. However, in contrast to binding to HPV-18, the binding affinities of the two BPV-1 complexes are very similar to one another. It has been suggested that these differences in relative affinities are associated with changes in the width of the minor groove between the BPV-1 and HPV18 complexes (Hizver et al., 2001), but accounting for subtleties of this type clearly poses further theoretical challenges.

The use of structural information to understand protein-DNA binding specificity and to identify TF binding sites will undoubtedly see rapid progress in the coming years. The paper of Rohs et al. (2005) is likely to be an important step in the development of approaches of this type because it offers a means to describe efficiently the conformation of the unbound DNA molecule in atomistic terms and hence to improve treatments of the energetic effects of indirect readout. It will be necessary to develop methods to calculate strain energies for the conformations that are generated. In addition to the direct use of all-atom force-fields for this purpose (Paillard and Lavery, 2004), a number of papers have used the structure-derived parameters of Olson et al. (1998) to calculate deformation energies. The structure-based prediction of TF binding sites must account for direct readout effects as well, and the energy-based analysis of known TF binding sites is an essential step in this direction. In addition, much has already been learned from experimental and statistical studies of known TF sites (Bulyk, 2003), and the integration of this information with physical-chemical insights promises to become a new and exciting research area (see e.g., Sarai and Kono, 2005). Indeed we will need to learn the lessons of many diverse approaches if we are to fully understand the subtle differences in protein-DNA binding specificity that underlie transcriptional regulation.

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YjjX: From Structure "Tu" Function

Zheng et al. (2005) show by structural analysis that YjjX, a hypothetical protein in *E. coli*, is an ITPase/XTPase and suggest that it may play dual roles in prokaryotic translational regulation and oxidative cell stress response.

As the number of structures deposited in the Protein Data Bank grows and the protein-fold space is filled, structural biology is becoming an increasingly powerful tool for predicting or annotating the function of proteins. For instance, structural genomics projects are aiming to characterize the structure and function of each gene product identified at the genome level. A substantial fraction of the sequenced genes encode proteins of unknown or hypothetical function. These functionally unknown proteins share little sequence similarity with previously characterized proteins, and, in such cases, traditional prediction methods for inferring the function of a protein, including sequence analysis and fold prediction, frequently do not work. A paper in this issue of *Structure* (Zheng et al., 2005) describes the three-dimensional structure of YjjX, a protein from *Escherichia coli*, and efforts to unambiguously reveal its previously unknown function.

Prior to the structural characterization of YjjX, there were very few pointers toward its function. A PSI-BLAST search highlighted other proteins with unknown function, and only one report implicating YjjX in thiamine metabolism had previously been published (Lawhorn et al., 2004). Once the structure was determined to 2.25 Å resolution by Zheng et al. (2005), clues for the function of YjjX began to fall into place following comparisons with two similar structures, Mj0226 from *Methanococcus jannaschii* (Hwang et al., 1999) and Maf from *Bacillus subtilis* (Minasov et al., 2000). Both are

nucleotide binding proteins, suggesting that YjjX could have a similar function. Identification of the putative active site of YjjX and comparison with the active site of Mj0226, a nucleotide phosphatase (NTPase), led the authors to hypothesize that YjjX might also have NTPase activity. Although the authors did not unambiguously identify the location of the active site or the essential nucleotide binding residues, their kinetic assays using commonly available nucleosides as substrates appear to confirm that YjjX does indeed possess NTPase activity.

A central paradigm has been that RNA transcription does not possess a proofreading mechanism, unlike DNA replication. In light of this, a particularly interesting result from the kinetic assays performed by the authors indicates that YjjX can hydrolyze inosine triphosphate (ITP) and xanthosine triphosphate (XTP) approximately 30–100 times faster than other nucleoside and deoxynucleoside triphosphates.

ITP and XTP are produced by deamination of ATP and GTP in response to exposure to chemical mutagens and to oxidative damage, and they reduce both the rate and efficiency of transcription. Furthermore, ITP incorporation in elongation complexes has a detrimental effect on the stability of the DNA/RNA hybrid (Shaevitz et al., 2003), while dITP incorporation inhibits further nucleotide addition by human RNA polymerase II (Thomas et al., 1998). As a consequence, mechanisms are required in nucleic acid synthesis to safeguard against undesired incorporation of ITP and XTP. The role of YjjX as an ITPase/XTPase, therefore, might be to prevent or minimize incorporation of undesired nucleotides ITP and XTP into RNA, although this remains to be confirmed by further study.

A second interesting aspect of the study by Zheng and colleagues (Zheng et al., 2005) is the association of YjjX with the elongation factor Tu (EF-Tu). One important approach to determine the function of any protein is to identify its interaction or binding partners. From their GST pull-down experiments, the authors found that YjjX associates with EF-Tu, a prokaryotic peptide elongation factor that is responsible for escorting aminoacyl-tRNAs to the A site on the ribosome and for translocation of the ribosome along the mRNA. Cells trigger certain protective mechanisms to slow or stop translation when they experience stresses such as oxidative stress or antibiotic treatment. EF-Tu can additionally serve as a chaperone for unfolded and denatured proteins in *E. coli* (Kudlicki et al., 1997), and phosphorylation of EF-Tu has been shown to inhibit transcription elongation by preventing formation of the EF-Tu/GTP/aminoacyl-tRNA ternary complex (Cashel et al., 1996). The association of YjjX with EF-Tu suggests that it could play a role as a cell-protective enzyme. Indeed, the authors postulate that YjjX might interact with EF-Tu/GTP or EF-Tu/GDP to hinder the formation of the EF-Tu/GTP/aminoacyl-tRNA ternary complex.

While this study is a nice example of the power of structural biology as a tool for determining the function of a protein, a number of important questions still remain to be addressed. Why, for instance, is YjjX selective for ITP and XTP? In fact, it is fascinating that ATP, GTP, and TPP are competitive inhibitors of the ITPase activity of YjjX. It seems likely that understanding this mechanism of inhibition will have to await further structural studies. Given that EF-Tu functions as a chaperone, further work is needed to characterize this interaction between YjjX and EF-Tu and to establish its functional relevance. This, together with the possible functional roles of YjjX in preventing incorporation of undesirable nucleotides into RNA and as a cell-protective enzyme, is a significant area for further study. The role of YjjX in cell protection is consistent with a previous study in which YjjX was implicated in detoxification of bacimethrin and 4-amino-2-trifluoromethyl-5-hydroxymethylpyrimidine in the cell (Lawhorn et al., 2004). The answers to these questions should provide insights into translational regulation in prokaryotes in general and, more specifically, into mechanisms that safeguard against incorporation of undesirable noncanonical nucleotides into RNA.

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