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Structural Insights into plasticity of DNA-protein interactions in tetracycline receptors

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Tetracycline receptors (TetRs) are one of the most prevalent classes of transcription factors that are ubiquitously present in several antibiotic resistant pathogens, where they control efflux of antibiotics out of the cell. In particular, bacteria of the streptomyces genus that produce 70% of the commercially available antibiotics are uniquely placed as they possess mechanisms to not only resist antibiotics but also have the ability to produce them. Hence, TetRs from these species are the likely the evolutionary progenitors of this superfamliy. Here, we present a series of X-ray structures of two TetR proteins CprB and TylP from streptomyces coelicolor and fradiae, respectively that are putative gamma butyrolactone receptors. The structure of CprB-DNA complex reveals that unlike tetracycline receptor, TetR, these proteins bind to DNA as dimer of dimers[1]. This mode of binding is analogous to the multidrug efflux regulator QacR from Staphelococcus aureus. A comparative analysis of the structures of CprB with a consensus DNA sequence and its biological operator sequence in conjunction with fluorescence lifetime, anisotropy decay kinetics and molecular dynamics help entail the mechanism of action [2,3]. Specific changes in protein-DNA interactions that occur on binding of the different DNA sequences with CprB bring forth the plasticity that exists at this interface. Moreover, the role of the extended positively charged arm, commonly found in these species is entailed to enhance DNA binding by establishing additional contacts with the minor grove of the DNA[3]. Comparison of the structure of TylP with CprB brings out the diversity and the striking difference in the mode of regulation of the proteins across species. Structural analysis reveals that TylP appears to be more tightly regulated than CprB as it possesses a unique 25 amino acid long helix-loop extension that clasps the DNA binding motif thereby, sequestering it in a conformation that prevents it from binding to DNA.

The dimeric interface in these TetRs also plays a crucial role in allostery. It is along this interface that pendulum like motions occur that assist in sampling the conformational space, facilitating switch between the DNA and the ligand bound states. Analysis reveals that this interface is completely different in the two proteins. Helices alpha 8 that participate in dimerization are oppositely direct in the two proteins. In both cases a 'V' shaped interface is created. However, in CprB the top is anchored while in TylP the bottom is where the boundary is strengthened. Additionally, TylP has an extra butterfly shaped motif that partakes in inter-subunit interaction. Therefore, the structure clearly highlights that both CprB and TylP have marked differences in their mode of regulation and have evolved to respond to their respective species specific and pathway specific requirements. Overall, the structures presented here help in developing broader insights into mechanism of regulation by TetR regulators.

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