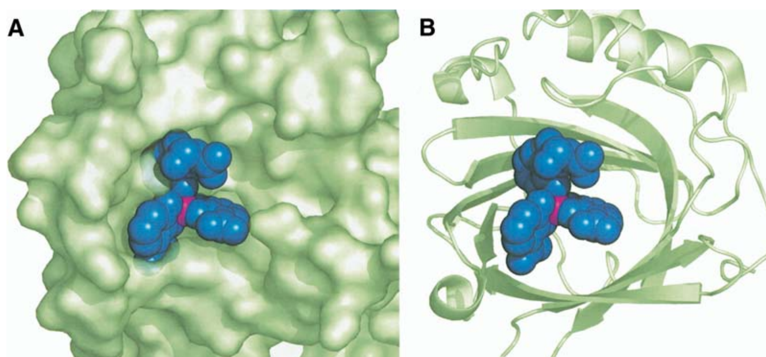

Figure 2. The Tripartite Binding Cavity of Siderocalin with Ferric Enterochelin Hydrolysis Products (Blue)

The Fe is in pink and protein (1L6M) is in white (A, surface rendering; B, backbone illustration).

same three subsites host specific parts of the asymmetric carboxymycobactins.

Perhaps even more remarkable than this broad specificity is the fact that it is not a consequence of a malleable binding site that adjusts itself to accommodate different ligands; only two amino acids (of the ~25 that line the cavity) are observed with different rotamer conformations in the various siderocalin-siderophore structures described. This is in stark contrast to the structural basis for the "directed promiscuity" of the pregnane X receptor (PXR) (Watkins et al., 2001, 2003), the binding site of which can expand or "breathe" to adapt to different ligands. Thus PXR is able to upregulate the expression of drug-metabolizing enzymes in response to the binding of structurally diverse xenobiotics. In the PXR isolated ligand binding domain, a single ligand can be observed in three distinct orientations. In contrast, although the siderophores are not tightly fixed in the siderocalin binding site (as judged from the appearance of the electron density), they are trapped in a single orientation primarily by electrostatic and cation-pi interactions.

Given the large number of sequences that are associated with the lipocalin superfamily, and the fact that for many of these proteins a function remains to be ascribed, the work of Holmes and colleagues (2005) suggests new avenues to be considered for function, and is another remarkable illustration of just how different the lives of members of the same superfamily can be. At the same time, it is a sobering reminder of the complexity of the task of inferring biological function from structure.

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Oxygen, Metabolism, and Gene Expression: The T-Rex Connection

In this issue of *Structure*, Sickmier et al. (2005) report the structure of the redox-sensing repressor from the gram-positive bacterium *Thermus aquaticus* (T-Rex), a protein that links gene expression to oxygen limitation and the metabolic state of the cell.

Organisms from microbes to humans have evolved a variety of enzymes to adjust their metabolism to fluctuations in oxygen availability. However, we are only beginning to understand the molecular mechanisms by which organisms detect changes in the oxygen levels of their environment and modulate the expression of metabolic genes. Cells are faced with two alternatives for sensing oxygen levels. One option is to directly sense intracellular levels of oxygen. This is exemplified by hypoxia-inducible factor (HIF), a transcriptional activator of genes important for both acute and chronic responses to low oxygen in higher eukaryotes. HIF is active under conditions where oxygen is limiting, but in the presence of abundant oxygen, a proline residue is hydroxylated resulting in polyubiquitination and proteosomal destruction of the HIF protein (Ivan et al., 2001).

The *Escherichia coli* transcription factor FNR, which serves as both a transcriptional activator and repressor of respiratory genes, is also directly regulated by oxygen levels (Lazazzera et al., 1996). Under anaerobic conditions a 4Fe-4S cluster maintains the protein as a functionally active dimer. In the presence of oxygen, the 4Fe-4S cluster rapidly converts to a 2Fe-2S form resulting in monomeric and inactive FNR. A second option is to respond to the metabolic consequences of perturbations in oxygen levels. This type of regulation is exemplified by the *E. coli* ArcAB two-component system, which also modulates the transcription of respiratory genes (Georgellis et al., 2001). Under aerobic conditions, ArcB kinase activity is directly inhibited by the oxidized form of quinone electron carriers. Under limiting oxygen conditions, the cellular levels of oxidized quinones decrease leading to activation of the ArcAB signaling pathway.

Until the discovery of the *Streptomyces coelicolor* redox-sensing repressor (Rex), little was known about how most Gram-positive bacterial species regulate gene expression in response to limiting oxygen (Brekasis and Paget, 2003). Brekasis and Paget began investigating the mechanism by which *S. coelicolor* responds to oxygen deprivation by focusing on the *cydABCD* operon, which was known to be induced by oxygen limitation in other bacterial species. Upon identifying a *cydABCD* promoter that was induced by low oxygen, they noted an inverted repeat DNA sequence (ROP–Rex operator) that could potentially serve as a transcription control site. Genome-wide searches for the ROP sequence resulted in the identification of ROP sequences upstream of other respiratory operons, including an operon encoding an uncharacterized DNA binding protein later renamed Rex. Genetic and biochemical experiments showed that Rex was necessary for *cydABCD* repression under aerobic conditions and could directly bind the ROP site. Interestingly, Rex also contained a dinucleotide binding domain commonly found in NAD^+-dependent dehydrogenases. Biochemical experiments investigating the DNA binding properties of Rex revealed that the DNA binding protein tightly interacted with the ROP site in the presence of NAD^+. In contrast, low micromolar concentrations of NADH caused Rex to dissociate from the ROP sequence. Further experiments showed that NAD^+ competes with NADH for Rex binding. Taking into consideration that NADH/NAD^+ ratios have been found to increase in oxygen-limited *E. coli* cultures, Brekasis and Paget proposed that the DNA binding activity of Rex is regulated in response to oxygen levels via NADH/NAD^+ redox poise.

In the present study, Sickmier et al. (2005) provide the molecular basis for NADH-dependent allosteric inhibition of the Rex-DNA interaction. The authors determined the structure of the *T. aquaticus* version of Rex (T-Rex), which exhibits the same functional characteristics as its *S. coelicolor* homolog. The domain topology of NADH-bound T-Rex is similar to other NAD^+-dependent hydrogenases in that it contains two domains; in the case of T-Rex, a winged-helix DNA binding domain and a dinucleotide binding domain (Figure 1). Three structural observations provide insight into the mechanism by which NADH/NAD^+ exchange alters the DNA affinity of dimeric T-Rex. First, the two winged-helix DNA binding domains are oriented in such a way that would not allow T-Rex to bind the ROP site, explaining why NADH binding results in Rex dissociation from DNA. Second, the dinucleotide binding site is located at the dimer interface of T-Rex, with the nicotinamide rings of NADH buried between the domains. In contrast, the NAD^+ is bound between the substrate binding domain and dinucleotide domain in NAD^+-dependent dehydrogenases such as the *C*-*t*erminal *B*inding *P*rotein (CtBP) (Figure 1) (Kumar et al., 2002). In T-Rex, the exchange of NADH for NAD^+ would likely have dramatic effects on the dimer interface resulting in reorientation of the wing-helix DNA binding domains. A similar conformational change is thought to occur in the DNA binding domains of the transcription factor OxyR upon disulfide bond formation (Choi et al., 2001). Third, the C-terminal α-helix of each T-Rex monomer inserts between the winged-helix domain and dinucleotide do-

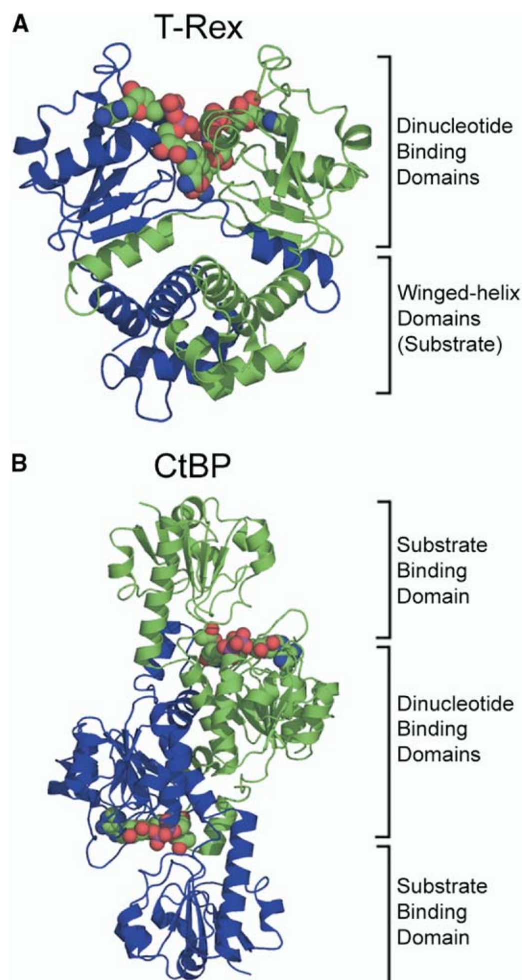

Figure 1. Structural Comparison of T-Rex and CtBP

(A) Structure of the T-Rex dimer with the C-terminal domain swapped α helices located between the dinucleotide binding domains and winged-helix domains.

(B) Structure of the human CtBP dimer, a NAD-dependent transcriptional regulatory protein, with the NAD molecules located between the dinucleotide binding domains and substrate binding domains. The NADH molecules are displayed in space-filling style.

main of the opposing monomer. Since the C-terminal α-helix is connected to a loop that directly contacts the nicotinamide ring, the helix could serve as the lever that allows the DNA binding domains to reorient in response to NADH/NAD^+ exchange.

While the structure of NADH bound T-Rex adds new insight into NADH/NAD^+ regulation of gene expression, it raises new questions as well. What type of conformational change occurs upon NADH/NAD^+ exchange, what is the role of the C-terminal α-helix, and what are the NADH and NAD^+ dissociation constants? It has been reported that CtBP, which functions as both a dehydrogenase and a eukaryotic transcriptional corepressor, as well as the NPAS/BMAL transcription factors also are regulated by dinucleotide ratios (Rutter et al., 2001; Zhang et al., 2002). How many other proteins are modulated by fluctuations in NADH/NAD^+ ratios, and what other conformational changes are brought about by dinucleotide binding? Did even Tyrannosaurus Rex contain a T-Rex protein? Finally, two critical questions in all organisms with putative NADH/NAD^+ sensors are: how do the concentrations of free NADH and NAD^+ compare to the binding affinities of the sensor proteins and how do NADH/NAD^+ ratios change in different oxygen environments, cells types, and subcellular compartments? It is likely that Rex will serve as a paradigm for answering many of these questions.

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