THE CRYSTAL STRUCTURE OF YODA, AN *E. COLI* PROTEIN INVOLVED IN HEAVY METAL STRESS

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Heavy metals, such as mercury and cadmium, are very toxic in living organisms, which have therefore evolved various defence and control mechanisms. Even for metals that are essential for the correct functioning of living organisms, their presence within the cells has to be tightly controlled to avoid negative effects. In most cases, and certainly in the case of cadmium, the actual toxic effect is in part due to the oxidant properties of these metals.

We have solved the structure of YodA, a novel protein implicated in cadmium stress is *E. coli*. This protein has been suggested as a member of a new family of cadmium-response proteins in bacteria (1). While there is no sequence similarity to proteins with known folds, the three-dimensional structure shows that YodA is a memeber of the lipocalin/calycin family. At the same time, we show that YodA is a metalloprotein, with a high-affinity site for divalent cations such as zinc, nickel and cadmium.

We shall describe the structure of the protein and propose hypotheses for its function in bacteria.

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STRUCTURAL BIOLOGY OF 14-3-3 PROTEINS

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14-3-3 proteins were the first signaling proteins to be identified as discrete phosphoserine/phosphothreonine binding molecules. These proteins play an important role in the regulation of signal transduction, apoptosis, cell cycle control, and nutrient-sensing pathways [1,2]. The 14-3-3 proteins are a conserved family of acidic proteins (molecular mass ranging from 27 to 32 kDa) present in high abundance in all eukaryotic organisms studied so far. Many organisms express multiple isoforms; for example, in mammals seven isoforms have been identified. All 14-3-3 isoforms can form stable homo and hetero-dimers. Though 14-3-3 proteins perform different functions for different ligands, general mechanisms of 14-3-3 action include changes in activity of bound enzymes, control in sub-cellular localization of 14-3-3 bound proteins, and alterations in protein-protein interactions of bound ligands with other proteins.

Crystal structures of human 14-3-3 zeta and tau isoforms, and structures of 14-3-3zeta bound to various peptides representing 14-3-3 binding motifs provided first structural insight into understanding of the biological function of 14-3-3 proteins [3,4]. These structures illustrate the conserved fold of the 14-3-3 proteins, where each monomer is composed of nine antiparallel a-helices, and two monomers form cup-shaped dimers with a large deep channel in the center running the length of the dimer. The walls of the channel contain amphipathic grooves that are ~30 A long, and residues lining the grooves are mostly conserved among the different isoforms. Phosphoserine-containing peptides were observed to bind in an extended conformation within these grooves. Recently, the structure of 14-3-3zeta bound to an enzyme serotonin N-acetyltransferase in complex with a bisubstrate analog, was solved [5]. This structure allowed to describe how 14-3-3 interacts with an enzymatically active protein - 14-3-3 stabilizes the conformation of an adjacent region in the enzyme, causing enhanced substrate binding and product formation.

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STRUCTURE OF THE PLECTIN ACTIN BINDING DOMAIN

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Plectin and its isoforms are versatile cytolinker proteins of very large size (molecular mass over 500 kDa) that are expressed in a wide variety of mammalian tissues and cell types. Biochemical data indicate that plectin plays an important role in cytoskeleton network organization and regulation, with consequences for viscoelastic properties of the cytoplasm and the mechanical integrity and resistance of cells and tissues. Defects in plectin genes cause autosomal recessive or dominant hereditary diseases, characterized by severe skin blistering with or without muscular dystrophy. Plectin has been well characterized biochemically and genetically. Electron microscopy revealed that the protein has a dumbbell-like structure com-