02.X-5 THE trp REPRESSOR/OPERATOR SYSTEM: THE CRYSTALLOGRAPHY OF LIGAND-ACTIVATED GENETIC REGULATION. By R. W. Schevitz, A. Joachimiak, R.-G., Zhang, C. L. Lawson, R. Q. Marmorstein, Z. Otwinowski, and P. B. Sigler, Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois

We have solved and refined the crystal structures of the "active" trp repressor and the unliganded "inactive" trp aporepressor to 1.4 A and 1.8 A respectively. [Repressor: $P3_121$: a = b = 50.3 A, c = 73.6 A; one subunit of 107 amino acids per asymmetric unit, d_{min} > 2.2 A and $P2_12_12$, a = 53.62 A, b = 53.24 A, c = 33.03 A, one subunit per asymmetric unit, d_{min} > 1.6 A. Aporepressor: $P2_12_12$, a = 44.50 A, b = 57.40 A, c = 34.20 A, one subunit per asymmetric unit.] By contrasting the two models we can visualize directly the structural basis for the activating transition caused by binding tryptophan and infer from these conformational changes which features are likely to be important in operator binding.

Both molecules are symmetrical dimers in which the subunit interface is formed by an unusual arrangement of interlinked α-helices. This unique and extensive interface forms a unified and solid globular domain that resists tertiary and quaternary structural changes and thereby prevents cooperativity upon tryptophan binding. Flanking the rigid central core are two flexible 'DNA-reading heads' that contain the bihelical motif found in other DNA-binding bacterial regulatory proteins. The reading heads are molded by bound tryptophan to a shape that complements the surface of the operator's major groove. The tryptophan binding sites are wedged between the solid central core and the flexible reading heads. The flexibility of the reading heads depends on a hinge composed of a tandem of unusually small side-chains in the 'turn' of the bihelical domain and explains why the mutational change of Ala ⟶ Val 77 freezes the protein in the active state.

The complimentary recognition surface between trp repressor and its cognate operator is primarily nonpolar, with specific hydrogen bonds playing an apparently subordinate role. The snuggly fitting complementary surfaces of this protein/nucleic acid complex exclude solvent and counter-ion thereby amplifying an already strong electrostatic attraction only at the operator site.

Progress will be reported on the structure analysis of: (1) pseudorepressor, an inactive near-isomorph of repressor formed by binding competing tryptophan analogues and (2) monoclinic repressor/operator cocrystals ($P2_1$, a = 44.4 A, b = 74.0 A, c = 106.4 A, β = 95.7 degrees, two complexes per asymmetric unit, d_{min} > 2.3 A).

02.X-6 SEQUENCE-DEPENDENT DNA-RECOGNITION; CRYSTALLOGRAPHIC STUDIES OF DNaseI: OLIGONUCLEOTIDE COMPLEXES. By A. Lahm and D. Suck, Biological Structures Division, EMBL, Heidelberg, West Germany.

Interactions of proteins with double-stranded DNA vary from being strictly sequence-specific to being completely unspecific. Examples of the former are the interaction of repressors with operator sequences or of restriction endonucleases with their cognate recognition sequences; an example of the latter is the histone-like DNA binding protein II from bacteria. Structural models have been proposed for either class and will be discussed briefly. A third class of proteins with properties in between these two extremes bind to DNA in a sequence-dependent, rather than a sequence-specific or completely random manner. These proteins somehow recognize sequence-dependent variations in DNA conformation. Bovine pancreatic DNaseI belongs to this group of proteins and accordingly shows a sequence-dependent DNA-cutting pattern. Based on the 3D-structure of DNaseI we have proposed a model for binding to and cutting ds-DNA. Subsequently, we have succeeded in co-crystallizing a series of self-complementary octanucleotide duplexes with DNaseI. HPLC-analysis of the dissolved crystals showed that the bound oligonucleotides were partially degraded in a sequence-dependent manner. 1.9Å data have been collected for one of these complexes and its structure was solved by the molecular replacement method using the refined DNaseI co-ordinates, and observed amplitudes to 3Å resolution. A piece of DNA was built into the resulting difference density and is presently being refined. At the present state of the analysis it is clear that the oligonucleotide adopts a somewhat distorted B-type DNA conformation. In agreement with the predicted model (Suck and Oefner, Nature, 1986, 321, 620-25) an exposed loop of DNaseI interacts in the minor groove of B-DNA and electrostatic contacts are formed between several Arg and Lys residues with phosphates of both DNA strands across the minor groove. The mechanism of sequence-dependent cutting of ds-DNA by DNaseI will be discussed on the basis of the 3D-structure of the complex.