02.4-03 CRYSTALLOGRAPHIC STUDIES OF METAL BINDING TO TRANSFER RNA. By J. R. Rubin and M. Bendersdorrngom, Department of Biochemistry, University of Wisconsin, Madison, WI 53706

The role of cooperatively bound cations in the stabilization of biologically active conformers of tRNA is well established. Previous crystallographic studies on the magnesium form of phenylalanine tRNA from yeast have shown the presence of three to four tightly bound magnesium ions located in the tertiary structural folds of the molecule. As a continuation of our structural studies on tRNA and its interactions with biologically important ligands (Liebman et al., Proc. Natl. Acad. Sci. USA, 72, 4821 (1977)), we have now examined the binding of a series of transition metal ions and complexes to crystalline phenylalanine tRNA by x-ray crystallography.

Zinc salts have been shown to substitute for magnesium in the enzymatic aminoclylation of tRNA. A crystalline complex of Zn(II)-phe tRNA prepared by soaking native phe tRNA crystals in ZnCl2 solutions shows at least five strong binding sites for zinc on the molecule. The two major zinc binding sites correspond to magnesium binding sites in the structure. All but one of the zinc binding sites involves direct coordination to N7 of guanine bases, including G20, G15, G63 and G43. Similar studies on the binding of other metals e.g. Cd++, Pb++, Ni++, Cu++ etc. are in progress. The effects of such metal ion binding on the conformations of tRNA will be discussed.

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02.4-04 POLYMORPHISM IN DNA-RNA HYBRIDS. By Struther Arnott, R. Chandrasekaran, A. Banerjee, A.G.W. Leslie and E. C. Selsing, Department of Biological Sciences, Purdue University, West Lafayette, IN 47701, U.S.A.

DNA-DNA duplexes are quite polymorphic with a range of helical structures in which the average axial translation per nucleotide 0.26 nm ≤ h ≤ 0.38 nm and the corresponding rotation 50.0° ≤ θ ≤ 48.0° are both quite variable (Leslie et al., J. Mol. Biol. (1980) 143, 49-72; Arnott, Biophys. J. (1980) 32, 249-250). DNA-RNA helical duplexes are much less variable: all known allomorphs have qualitatively similar conformations although 0.26 nm ≤ h ≤ 0.31 nm, 30.0° ≤ θ ≤ 52.7°.

Our systematic fiber diffraction analyses of poly dN-poly dR (where N = A, T or U, G or I, C and N = the Watson-Crick complement of N) have revealed that in general DNA-RNA hybrids are isomorphous with A-DNA (Fuller et al., J. Mol. Biol. (1985), 12, 60-80) in less hydrophilic environments, but isomorphous with α-RNA (Arnott et al., Nature (1968), 220, 561-4) in more hydrophilic environments. In addition an unique duplex allomorph (h = 0.32 nm, θ = 36.0°) observed with poly d1-poly dC may have the hitherto unobserved conformation which was mistakenly proposed for B-DNA by Crick and Watson (Proc. Royal. Soc. A (1954), 225, 80-96).

02.4-05 POLYMORPHISM IN DNA-RNA HYBRIDS. A recognition complex between EcoRI endonuclease and a synthetic oligonucleotide (sequence CCGGATTGCGCC) crystallizes in space group P212 with unit cell parameters a = 129 Å, b = 128 Å, c = 47.3 Å. The presence of the DNA was confirmed by single crystal ultraviolet microbeam spectroscopy. The most likely asymmetric unit contents are one 31,000 dalton enzyme subunit and one strand of DNA, yielding a Vm of 3.41. This implies that the DNA-protein complex obeys two-fold rotational symmetry, which has been incorporated in the crystalline lattice. Large monoclinic crystals have also been obtained in the absence of DNA; their space group is C2 with a = 211 Å, b = 128 Å, c = 49 Å, and γ = 96.5°. Striking similarities between the lattices and diffraction patterns of these two forms have been noted. Structure analysis is in progress. Supported in part by NIH grants No. GM-25671, RR-07084 (JMR) and GM-25729 (PG).

02.5-01 CRYSTALLOGRAPHIC ANALYSIS OF EcoRI ENDONUCLEASE-DNA COMPLEX. By John M. Rosenberg, John Grable, and Christin Frederick, Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260; Patricia Greene, Dept. of Biochemistry, Univ. of Calif., San Francisco, CA 94113; Ketichi Itakura, City of Hope Medical Center, Duarte, CA 91010; Horace Drew, Dept. of Chemistry, CalTech., Pasadena, CA 91125; and Roberto Crea, Genentech, So. San Francisco, CA 94080.

A recognition complex between EcoRI endonuclease and a synthetic oligonucleotide (sequence CCGGATTGCGCC) crystallizes in space group P21 with unit cell parameters a = 129 Å, b = 128 Å, c = 47.3 Å. The presence of the DNA was confirmed by single crystal ultraviolet microbeam spectroscopy. The most likely asymmetric unit contents are one 31,000 dalton enzyme subunit and one strand of DNA, yielding a Vm of 3.41. This implies that the DNA-protein complex obeys two-fold rotational symmetry, which has been incorporated in the crystalline lattice. Large monoclinic crystals have also been obtained in the absence of DNA; their space group is C2 with a = 211 Å, b = 128 Å, c = 49 Å, and γ = 96.5°. Striking similarities between the lattices and diffraction patterns of these two forms have been noted. Structure analysis is in progress. Supported in part by NIH grants No. GM-25671, RR-07084 (JMR) and GM-25729 (PG).

02.5-02 STRUCTURE OF THE BACTERIOPHAGE LAMBDA CRO REPRESSOR: MODEL FOR PROTEIN-DNA INTERACTIONS. By W.P. Anderson, D.R. Ohlendorf, T. Takeda, B.M. Matthews, Department of Biochemistry, University of Alberta, Edmonton, Alberta, TS6 Z1N, Canada. Institute of Molecular Biology, University of Oregon, Eugene, Oregon, 97403, U.S.A. Chemistry Department, University of Maryland, Baltimore County, Catonsville, Maryland, 21229, U.S.A.

The bacteriophage lambda c-cro protein is a small (66 amino acids) polypeptide which binds to specific DNA base sequences in the lambda genome. We will present the three-dimensional structure of the c-repressor and its apparent mode of interaction with operator DNA. The postulated interaction between repressor and operator is consistent with a variety of chemical and genetic evidence, and may provide a general model for the interaction of proteins with helical DNA.

A principal feature of the proposed binding of c to DNA is the use of symmetry. The two-fold symmetry axis of the repressor coincides with the approximate two-fold symmetry axis of the DNA operator. This type of interaction is expected to be of general significance for proteins which recognize specific, sequence-specific regions of the DNA. Our results indicate that the symmetrical interactions are essential for specific DNA-protein interactions and are a consequence of the oligomeric structure of the regulatory proteins rather than the source of special type of DNA structure.

The proposed model places two-fold related α-helices in successive major grooves of right-handed B-DNA. The amino acid side chains extending from this helix would then provide the base sequence specificity of the c-repressor operator complex. Evidence for a similar structure in some other DNA binding proteins will be presented.