02.X-09 THE STRUCTURE OF THE NUCLEOSOME AND THE FOLDING OF CHROMATIN.

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Crystallographic Studies X-ray studies on a new crystal form of the nucleosome core, which diffracts to much higher resolution than before (Finch et al., Nature, 269, 24-36, 1977), show the form of the DNA double helix and also the overall dyad in the particle. An analysis by isomorphous replacement is in progress.

A neutron diffraction study of the crystals using the method of contrast variation has shown the general distribution of protein and DNA in the particle and has confirmed our earlier picture of the structure, in which $1\frac{3}{4}$ turns of a DNA superhelix of pitch 27 Å and radius 45 Å are wound around a wedge shaped protein core (with Lewit and Bentley, ILL, Grenoble).

Organisation of the DNA The distribution of diffuse intensity in the X-ray patterns shows that the DNA is not firmly fixed to the histone core all along its superhelical path, but only over limited regions. Their location correlates well with those regions of the DNA differentially protected against nuclease digestion.

The Histone Octamer The structure of the isolated histone octamer has been studied by electron microscopy combined with image reconstruction and a low resolution 3-D map obtained. On the basis of this, and chemical cross-linking studies, a proposal has been made for the spatial arrangement of histones in the nucleosome core, from which the roles of the individual histones in folding the DNA can be seen (with J.O. Thomas, Biochemistry Department, University of Cambridge).

Higher Order Folding and Histone H1 The role and location of the fifth histone H1 have been investigated by following with the electron microscopy the folding of chromatin, with and without H1, as a function of increasing salt concentration. It is concluded that H1 is located on the side of the nucleosome at the entry and exit points of the DNA, and stabilises the complete, two-turn 166 base pair particle, the structural element of chromatin (with Thoma & Koller, E.T.H., Zurich).

02.X-10 TOBACCO MOSAIC VIRUS: INTERACTION OF THE COAT PROTEIN WITH NUCLEOTIDES.

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Tobacco Mosaic Virus (TMV) is a long rod-shaped virus 180 Å in diameter. It has helical symmetry with $16\frac{1}{3}$ protein subunits per turn and the single-stranded RNA (~ 6400 nucleotides) follows the basic helix at about 40 Å radius. The protein subunits extend from 20 to 85 Å radius. There are three nucleotides bound to each protein molecule.

The complete virus particles of TMV do not crystallise but they can form extremely well oriented gels. Diffraction analysis of these has led to a model for the RNA configuration in which the 3 ribose sugars (found at 38, 38 and 45 Å radii) all have C3'-endo conformation and the 3 nucleotide bases form a claw-like structure around the left radial helix of the protein subunit. The corresponding three phosphate groups interact also with the neighbouring protein molecule from the turn below within the viral helix.

The protein disk of TMV, an obligatory intermediate in the assembly of the virus from its constituent RNA and protein is a polar aggregate comprising two rings each of 17 protein subunits. Crystals of the disk (mol. wt. = 600,000 per a.u.) have been analysed to 2.8 Å resolution, giving an atomic model for the coat protein. The subunit conformation within the disk is essentially the same as that in the virus except for the low radius flexible loop (residues 89-114), which is the only part of the protein extending inwards to lower radius than the RNA binding site. This loop is mobile without a defined conformation in the disk but its mobility is reduced on binding of oligonucleotides and it adopts a definite conformation in the virus in the presence of RNA. The flexibility of this loop allows it to be poised for interaction with, and intercalation of, RNA between adjacent layers of protein subunits during the process of viral assembly.

In order to study RNA binding to the disk (and thus the initial interactions during assembly), oligonucleotides have been allowed to diffuse into the disk crystals. While high concentrations mimic the assembly and cause dislocation, at lower concentrations the crystals remain intact. Initial studies with a specific trimer AAG and a purine-rich hexamer produced smaller changes in the crystals than those now observed on binding of a specific hexamer AAGAAG but in all cases distinct movements can be seen in some of the helices of the protein subunit together with a stiffening of the ends of the flexible loop.