02.3-04 A STUDY OF MICROTUBULE SELF-ASSEMBLY BY MEANS OF TIME RESOLVED X-RAY SCATTERING. E.M.Mandelkow[+],E.Mandelkow[+] and J.Bordas[§] (+)Max-Planck-Inst. for Medical Research, Heidelberg,(§)European Molecular Biology Lab., DESY,Hamburg,FRG.

Microtubules are protein fibers involved in the control of shape and motility of cells.They achieve this purpose by assembly and disassembly from their subunits (tubulin and MT-associated proteins,MAPs).These processes can be induced "in vitro"by warming the protein solution from 0°C to 37°C and vice versa.The experiments were carried out at the X-13 instrument in EMBL (See Bordas and Koch,these Proceedings and ref. therein).The technique employed yields information about the state of aggregation, particle shapes,subunit arrangements and kinetic parameters,(Mandelkow,Harmsen,Mandelkow and Bordas,Nature 287,595,1980).

The scattering of the cold solution arises from ring shaped aggregates of 36 nm diameter,tubulin subunits and other species.The warm solution contains microtubules of 24 nm diameter.The stages of assembly may be classified as pre-nucleation,nucleation,growth and post-assembly events.There are six detectable states during assembly as well as in disassembly.Nucleation is probably based on oligomers of tubulin and MAPs rather than rings.Assembly requires higher temperature than disassembly,leading to a hysteresis-like behaviour.The observed lag period prior to MT assembly is mainly due to pre-nucleation events rather than nucleation itself. The process can be repeated until the exhaustion of GTP.The implication for models of MT assembly will be discussed.

02.4-01 TWO STRUCTURES OF YEAST $tRNA^{Asp}$ AT 3 Å RESOLUTION. By D. Moras, M.B. Comarmond, J. Fischer[+], R. Giegé and J.C. Thierry, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15, rue René Descartes, 67084 Strasbourg Cédex and [+]Institut Le Bel, Université Louis Pasteur, 4, rue Blaise Pascal, 67070 Strasbourg Cédex, France.

Yeast $tRNA^{Asp}$ crystallizes from high concentrated ammonium sulphate solutions in two, nonisomorphous and temperature dependent interconvertible crystal forms ; the transition between the two forms occurring at ~ 20°C. Two independent (3 Å resolution) electron density maps have been obtained from an M.I.R. X-ray analysis.

Analysis of the molecular model constructed from the electron density maps confirm for both forms of $tRNA^{Asp}$ the folding originally found in $tRNA^{Phe}$; major differences concern the conformation of the loops and the relative positioning of the acceptor and anticodon stems which are more open than in the $tRNA^{Phe}$ structure conferring to the molecule its boomerang like shape. Crystal packing in both forms involves self-complementary GUC anticodon interactions thus making the structure a tempting model of a tRNA interacting with messenger RNA on the ribosome.

02.4-02 THE CRYSTAL STRUCTURE OF YEAST $tRNA^{Phe}$ IN THE CUBIC FORM. By J.K. Nachman, J.L. Sussman, R.W. Warrant* and S.H. Kim*, Department of Structural Chemistry, Weizmann Institute of Science, Rehovot, Israel and *Chemistry Department, University of California, Berkeley, California USA.

It is still an open question if the three-dimensional structure of a polynucleotide chain is determined uniquely by its nucleotide sequence, as well as how much flexibility there is in its structure. Yeast $tRNA^{Phe}$ in the cubic form is radically different in its space group symmetry - $I4_132$ - both from the two very similar structures of monoclinic and orthorhombic yeast $tRNA^{Phe}$, and from all other tRNA molecules of known three-dimensional structure. By determining the crystal structure of $tRNA^{Phe}$ in this form, we hope to better understand the role played by the nucleotide sequence on the one hand, and by crystal packing forces on the other, in inducing changes in the three-dimensional structures of different tRNAs. We also expect to gain insight as to how nucleic acids pack in a condensed state - relevant to such questions as the structure of RNA in ribosomes or DNA in nucleosomes - by comparing the interactions observed in this crystal structure with those previously seen for other tRNA structures.

Since no suitable heavy atom derivatives have been obtained so far, we used the molecular replacement method to fit the known coordinates of orthorhombic $tRNA^{Phe}$ (J.L. Sussman, S.R. Holbrook, R.W. Warrant, G.M. Church and S.H. Kim, J. Mol. Biol. (1978) 123, 607) into the cubic unit cell. The rotational parameters were determined by using Crowther's fast rotation function (R.A. Crowther, in "Molecular Replacement Method" (ed. M.G. Rossmann), 1971, Gordon and Breach, N.Y., pp. 173-178), while the translational parameters by a fast translation function, based on an R-factor algorithm of Rae (A.D. Rae, Acta Cryst. (1977), A33, 423), combined with a program to check intermolecular contacts.

The rotational search was performed first on data of 10 to 28 Å and then on data of 8 to 18 Å, with a 5° grid in the three eulerian angles, producing maps with highest-to-next-highest-peak ratio of 1.35 and 1.9, respectively. In both cases the maximum was obtained for the same rotational parameters, corresponding to a molecule with the axes of the two major helical domains of the "L" almost parallel to the edges of the cubic unit cell. The translational search was performed on 12.5 to 20 Å resolution data with a 2 Å grid in all three directions, producing a map with a mean R-factor of about 59% and a minimum R-factor of 36%; all other minima were higher than 46%. Finally, an intermolecular contact search between the centers of each base, ribose and phosphate group was performed, which showed that at the position corresponding to the minimum R-factor in the translation search the molecule has no group contacts less than 3 Å and only 3 contacts closer than 5 Å, while all other minima for the translation search have more than 200 contacts below 5 Å.

We have begun to refine the model using CORELS, a constrained-restrained least squares program (J.L. Sussman, S.R. Holbrook, G.M. Church and S.H. Kim, Acta Cryst. (1977) A33, 800). An initial refinement of the whole structure allowing the 4 arms (stems and loops) of the cloverleaf to move as discrete units (with the stereochemistry between the groups restrained close to standard values) cause the R-factor to drop from 36% to 24% for the 12.5 to 20 Å data. The R-factor for all data to 4 Å resolution is 39%. We are now examining difference electron density maps based on this model.