the lengths of helical nuclei which seem to be common to all collagens. They show (*ibid.*, 3754) that the kinetics of renaturation of cross-linked chains bear out all the predictions of theory for three independent first-order processes—nucleation, helix growth and a slow annealing process. In *Ascaris* collagen, the native state is a disulphide-linked lattice of single chains, each folded on itself into a triple-helix.

FLUORESCENT LABELLING

Getting a Grip on tRNA

from a Correspondent

ONE of the most favoured techniques for disentangling the structure of transfer RNA is to label certain of its bases. But it is difficult to know just what goes where in the molecule. Labelling the bases by chemical modification, for example, is far from straightforward because several bases may be affected by any particular chemical agent. A more elegant handle to approach this problem is provided by a built-in label in the phenylalanine tRNAs of yeast, wheat and rat liver, all of which have a fluorescent base, "Y", adjacent to the anticodon loop.

Earlier this year, by measuring the energy transfer between the Y base and a chromophore attached to the 3′ end, Beardsley and Cantor (*Proc. US Nat. Acad. Sci.*, **65**, 39; 1970) showed that the distance from the anticodon to the 3′ end, where the amino-acid would be attached, is 24–30 Å. Two recent reports from Cantor's laboratory (*Biochemistry*, **9**, 3514, 3524; 1970) continue the exploration of the structure of yeast phenylalanyl tRNA through various measurements based on the fluorescence properties of Y and a bound dye, ethidium bromide, which also fluoresces.

They report that the fluorescence intensity of the phenylalanyl tRNA is enhanced two and a half times when magnesium is added to a solution containing the tRNA at 320 nm. This change is accompanied by a 5 per cent drop in the extinction coefficient of the tRNA at 260 nm, simultaneously with a similar change in the magnitude of the principal positive circular dichroism band. The fluorescence intensity of Y, as in the case of many fluorescent molecules, increases in nonpolar solvents. Thus the enhanced intensity as a function of the magnesium concentration is interpreted as a conformational change that tucks Y into a hydrophobic region of the tRNA molecule. That such a large change occurs in the fluorescence intensity compared with absorption or circular dichroism, makes the Y base a very sensitive probe for studying the conformation around the anticodon loop of this tRNA. Beardsley and Cantor have shown that this change is not due to the interaction of the magnesium ions with Y itself, for there are no such changes in its fluorescence when the tRNA molecule is digested with nuclease. There is a further decrease in fluorescence as a consequence of the increased contact of Y with water molecules.

This natural fluorescent probe has also been used to demonstrate directly that there is a specific interaction with polyuridylic acid in a solution free of ribosomes (Eisinger *et al.*, *Proc. US Nat. Acad. Sci.*, **65**, 638; 1970). They found a small blueshift in the fluorescence spectrum as well as a drop in the fluorescence intensity in the presence of U_5 as well as poly U, but not in the presence of poly C.

Further information can be obtained by observation of the nanosecond depolarization in the fluorescence of Y. Because the molecule is tumbling freely in solution, the rotational diffusion coefficients can be estimated by measuring the randomization of the fluorescence induced by polarized light at the excitation frequency. The interesting result is that Y is rotating faster than the whole tRNA molecule. It is not possible to deduce whether this is due to free rotation of the fluorescent group itself or to a relatively flexible attachment of the anticodon loop to the rest of the tRNA. There is, however, greater fluorescence anisotropy than expected for a completely freely rotating chromophore, indicating some hindrance in the motion of Y.

In their second report in *Biochemistry*, Cantor *et al.* report that the dye, ethidium bromide, which intercalates into DNA, binds to tRNA chiefly at one site in the presence of a low concentration of magnesium. From a consideration of the quenching effect that this dye molecule has on Y, they conclude that it is probably bound in the double helical region adjacent to the anticodon loop. From nanosecond depolarization measurements, they conclude that the data are consistent with a tRNA molecule that changes from a prolate ellipsoid with an axial ratio of 2–3 in the presence of magnesium to one with an axial ratio of 4–5 when magnesium is removed. An interesting incidental observation is that the tRNA can still be activated by the activating enzyme with the bound dye present.

NUCLEOTIDES

Fragments and Factors

from our Nucleic Acids Correspondent

SOMETIMES experiments are elegant and interesting even if they do not lead to entirely unambiguous conclusions. Two pieces of work recently reported in the *Journal of Biological Chemistry* display these symptoms markedly. Siddiqui and Ofengand (*J. Biol. Chem.*, **245**, 4409; 1970) have attempted to study the function of the modified nucleotide pseudouridylic acid (Ψ) in tRNA. They chose to examine the formylmethionine tRNA from *E. coli*, because it only contains a single Ψ residue (occurring in the common sequence GTΨC). Unlike most other nucleotides, Ψ can be cyanoethylated with the aid of the reagent acrylonitrile, affording a way to probe its function in the tRNA. Unluckily, this tRNA also contains the modified nucleotide 4-thiouridylic acid, which also reacts with acrylonitrile to yield the S-cyanoethyl derivative. Siddiqui and Ofengand circumvented this problem using large fragments of the tRNA obtained by digesting it with T_1 ribonuclease. The fragments were fractionated by chromatography on columns of DEAE-cellulose. In this way they partially purified a piece encompassing about a quarter of the tRNA molecule (from the 5′ end) containing the 4-thioU residue, and also a fragment—the remaining three-quarters of the tRNA—containing the Ψ. The two pieces could "stick" together, and subsequently accepted methionine in the normal way.

It was therefore possible to effect the cyanoethylation of each of the fragments separately, and assess the amino-acid-accepting activity of such modified fragments when recombined with the complementary unaltered pieces. When 4-thioU alone was cyano-

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