when added back, it binds to the core protein to yield an active enzyme with the native ribonuclease conformation. Thus the *S*-protein sequence is insufficient to define the native geometry, and in the presence of the exchange enzyme it behaves exactly as expected (or indeed as the laws of thermodynamics dictate): the disulphide bonds are randomized (105 pairings being in principle possible), and the resulting molecule no longer forms active enzyme with the *S*-peptide. At higher protein concentration, intermolecular bridges are formed, leading to the appearance of large aggregates. If the *S*-peptide is present all along, however, there is no disruption of structure. The native conformation can also be reached from the randomized state: randomized *S*-protein incubated with *S*-peptide and exchange enzyme regains half initial activity within the first hour. The same experiments also work with "*C*-peptide", which is an *S*-peptide deprived of seven residues at its C-terminus, but still able to combine with and reactivate the *S*-protein. This work affords a compelling demonstration of the specificity of the native state, and the strength of the driving force urging the chain into this conformation.

Another interesting study, involving the making and breaking of cystine bridges, is described by Price, Stein and Moore in one of a series of papers dealing with the properties of pancreatic deoxyribonuclease (*ibid.*, 929). This protein has a molecular weight of 31,000, and is thus more than twice as large as pancreatic ribonuclease, but has two disulphide bonds rather than four. The enzyme needs calcium (or other divalent ions) for activity, and these evidently function by stabilizing the active conformation, although the binding is not especially strong. In the absence of calcium, treatment of the native enzyme with a reducing agent, such as mercaptoethanol, causes the rapid reduction of both disulphide bonds. This is an unusual situation: in most proteins some or all of these bonds are inaccessible, and are attacked only in denaturing conditions. Another unusual feature is the seemingly high reduction potential of the thiols which are formed, for they remain almost fully reduced after 24 hours in contact with air. When calcium ions are added, however, there is immediate refolding and reoxidation, with a full return of activity.

The calcium protects only one of the disulphide bonds against mercaptoethanol, but reduction of the other, even when followed by carboxymethylation with iodoacetate, leaves the enzyme activity intact. This half reduced state can also be achieved by adding calcium to the fully reduced enzyme in the presence of mercaptoethanol. It is clear that one bond is much more labile than the other, for during reduction without calcium, loss of activity parallels the progress of the reaction, so that cleavage of the second bond must instantaneously follow that of the first, which is rate-determining. The same parallel behaviour occurs on reoxidation in the presence of calcium. Here then is a case in which the active conformation is defined not only by the primary structure but also by the presence of a ligand, the calcium ion, required for stability.

There are of course fairly numerous examples of reduction of only one of several disulphide bonds, and another has been reported by Shapira and Arnon (*ibid.*, 1026), who find that a single cystine may be reduced in papain, leaving the other two intact. As in ribonuclease, addition of mercury to this derivative produces a new bridge, of the type –S–Hg–S–, and the resulting protein is active and crystallizable.

NUCLEIC ACIDS

Ribosomal RNA

from our Cell Biology Correspondent

A GENERAL rule governing the organization of the genomes of true cellular organisms—eukaryotes—seems to be emerging from studies of the hybridization of ribosomal and transfer RNA to DNA. Apparently all eukaryotes have in each haploid genome between 100 and 1,000 copies of the genes specifying ribosomal RNA but relatively few copies of the genes for each species of transfer RNA. *Saccharomyces cerevisiae*, a yeast, is the latest organism to be added to the list of eukaryotes which have been studied by hybridization. Schweizer, MacKechnie and Halvorson (*J. Mol. Biol.*, **40**, 261; 1969) report that 0·8 per cent of the nuclear DNA hybridizes with 18*S* RNA from the small ribosome subunit, 1·6 per cent hybridizes with 26*S* RNA from the large ribosome subunit and a maximum of 0·08 per cent hybridizes with transfer RNA. Assuming that the total genome has a molecular weight of $1{\cdot}25 \times 10^{10}$ daltons, these hybridization data indicate that the yeast has about 140 ribosomal RNA genes and 320–400 *t*RNA genes. This redundancy is of the same order as that previously reported for species of amphibia, mammals, insects and higher plants. The only marked difference between the yeast and all these other organisms is the percentage of the total genome devoted to specifying ribosomal RNA: in yeast it is 2·4 per cent; in the other eukaryotes so far investigated it is in the range 0·1 to 0·3 per cent. This difference is no doubt a reflexion of the ratio of total RNA to total DNA in yeast, which is ten times higher than in most organisms.

Although there are up to 400 *t*RNA genes in the yeast genome, this only represents on average about six genes for each *t*RNA species. In *Drosophila* the corresponding figure is thirteen, whereas bacteria seem to have only one gene for each *t*RNA. The 1 : 1 ratio of 18*S* to 26*S* ribosomal RNA in yeast and mammalian cells is consistent with the idea that the 18*S* and 26*S* RNAs arise from a single precursor molecule.

In another paper in the same issue of the *Journal of Molecular Biology*, Pinder, Gould and Smith (page 289) report evidence of the conservation of the structure of ribosomal RNA during evolution. Using polyacrylamide gel electrophoresis, they have studied the pattern of fragments that are yielded by T_1 ribonuclease digestion of *r*RNA from a wide range of bacterial and eukaryotic species and have reached three general conclusions. First, the structure of ribosomal RNA has differentiated appreciably during evolution; it would, of course, be surprising if it had not. Second, the overall structure, as opposed to the nucleotide sequence, tends to be conserved during evolution. Third, there is no evidence of heterogeneity in the ribosomal population of any one species. Unfortunately, the electrophoretic technique resolves structural rather than sequence differences, so, although the complexity of the electrophoretic pattern of RNA fragments does not increase as the number of gene copies per organism increases, this does not prove that all ribosomal RNA in any one species has the same base sequence.