of protein, assuming equimolar amounts of all the proteins. The number resolved by two-dimensional polyacrylamide-gel electrophoresis is at least 26 for the small subunit and 36 for the large subunit of rabbit reticulocyte ribosomes. The near-complete resolution of the ribosomal proteins opens up new possibilities for both quantitative and qualitative analyses.

A qualitative survey of the proteins in ribosomes and ribosomal subunits from wide variety of organisms, e.g. birds and mammals, demonstrates a remarkable degree of similarity. Since the position of a protein in the two-dimensional electrophoretogram is dependent on both its net charge and its size, this broad similarity suggests that there is a strong evolutionary pressure to conserve the physico-chemical properties of the ribosomal proteins. However, certain proteins appear to be characteristic of the species of origin, e.g. a protein of 45000 molecular weight found in the large subunit derived from the ribosomes of either the liver or reticulocytes of the rabbit.

An additional advantage of the particular method of analysis is the use of sodium dodecyl sulphate in the second dimension, which provides an estimate of the molecular weight of all the separated proteins. Knowledge of the molecular weights plus the relative quantities of the protein is required in order to calculate the stoichiometry of the proteins in the ribosome. We have recently been investigating methods for determining the relative quantities of individual proteins separated in polyacrylamide-gel slabs, and our progress on this topic will be discussed in some detail.

We have examined the effect of washing ribosomes through solutions of different KCl concentrations and KCl/Mg²⁺ ratios on the removal of proteins, both adsorbed 'contaminants' and 'ribosomal' proteins. At the salt concentrations commonly used to produce active ribosomal subunits it appears that some proteins, which we define as 'ribosomal' proteins on the basis that they appear to be present in amounts of about 1 mol/mol of ribosomes in the intact ribosome, may be partially washed off. A few differences between ribosomes in different functional states, e.g. monoribosomes, polyribosomes, native and derived subunits, and ribosomes unfolded with EDTA, have been observed.

EDTA-dissociated polyribosomes and messenger ribonucleoprotein dissociated from ribosomes in the presence of EDTA and purified by zonal centrifugation have also been subjected to two-dimensional polyacrylamide-gel electrophoresis. Three proteins, with molecular weights of approx. 150 000, 90 000 and 60 000, have been found in association with the messenger and one, of molecular weight 37 000, has been found in association with 5S RNA. These results may be compared with those of other workers,

who found two proteins, of molecular weights 130 000 and 68 000, in association with the messenger (Lebleu *et al.*, 1971) and one, of molecular weight 45 000 (Lebleu *et al.*, 1971) or 35 000 (Blobel, 1971), in association with 5S RNA in rabbit reticulocytes.

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The Genes for Ribosomal Ribonucleic Acid

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The coding sequences for rRNA species are reiterated within the genome of all organisms except *Mycoplasma* (Birnstiel *et al.*, 1971a). There is a general relationship between redundancy of rRNA coding sequences and genome size (Birnstiel *et al.*, 1971a). In prokaryotes the 5S rRNA coding sequences are closely linked to the 16S and 23S rRNA coding sequences (Jarry & Rosset, 1970; Smith *et al.*, 1968), unlike eukaryotes, where they are not closely linked to the 18S and 28S rRNA coding sequences (Brown & Weber, 1968; Wimber & Steffensen, 1970; Alloni *et al.*, 1971).

In *Xenopus* the coding sequences for 5S rRNA are 10 000–24 000-fold redundant (Birnstiel *et al.*, 1971b; Brown & Weber, 1968; Brown *et al.*, 1971) and are clustered within the genome (Brown & Weber, 1968), which has allowed their isolation as a pure DNA satellite (Brown *et al.*, 1971) in a manner similar to the isolation of the 18S and 28S ribosomal DNA (Birnstiel *et al.*, 1966). The isolation in pure form of a second repetitive DNA coding for an rRNA has allowed the construction of a general scheme for the organization of the repeating unit, consisting of non-coding spacer DNA alternating with the coding DNA sequence, for this class of repetitive DNA in eukaryotes. It remains to be seen whether this applies to tRNA and mRNA coding sequence clusters as well.

The assertion that reiterated coding sequences, such as those for rRNA, are all identical within a species has been debated for several years (Birnstiel *et al.*, 1971a). Nucleic acid molecular hybridization experiments have been used to support this idea (Birnstiel & Grunstein, 1972) and to deny it (Moore & McCarthy, 1968). However, the exact significance of the effects of the type and level of base substitutions on the rate of formation (Southern, 1971; Sutton & McCallum, 1971) and the thermal stability

(Laird *et al.*, 1969; Uhlenbeck *et al.*, 1971; Wells *et al.*, 1970) of hybrids is only just becoming apparent.

A more direct approach to the problem of heterogeneity within repeated coding sequences is the determination of nucleic acid sequences (Brownlee & Sanger, 1967; Southern, 1970). These techniques have successfully been employed to determine heterogeneity in the 5S RNA coding sequences of *Escherichia coli* (Brownlee *et al.*, 1968; Jarry & Rosset, 1971), but there is no unequivocal report of sequence heterogeneity in an RNA species from a eukaryote. On the contrary, the 5S RNA species from man (Forget & Weissman, 1969) and mouse (Williamson & Brownlee, 1969) are known to be identical, implying high evolutionary stability. E. M. Southern and I have shown that in *Xenopus* there are at least three and possibly five different 5S RNA sequences. The differences are not due to polymorphism in the population, nor to variable end group, which might occur on precursor molecules such as in *E. coli* (Monier *et al.*, 1969). There is tissue-specific expression of the different sequences, in that only one appears to be expressed in kidney cells but all are found in total RNA from ovary. The ovary unique 5S RNA sequences have been shown to be incorporated into ribosomes; however, it is not proven that they are functional.

These observations have clear implications for ideas about the evolution and organization of repeated coding sequences, the control of genetic activity and the tissue specificity of ribosomes in eukaryotic organisms.

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Ribosome Formation in Yeast

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The synthesis and processing of rRNA both in bacteria and in eukaryotic cells has been the subject of considerable investigation (for reviews see Darnell, 1968; Attardi & Amaldi, 1970).

The formation of mammalian rRNA involves a complex multi-stage maturation process. The two major rRNA components derive from a common large precursor molecule containing the sequences of one molecule of each of both species of mature rRNA and some excess of non-ribosomal RNA that is discarded during the maturation process. In bacteria each of the two species of rRNA is synthesized as an independent unit that is probably somewhat larger than the corresponding mature rRNA.

Yeast, a simple eukaryotic protist, resembles in certain aspects both animal cells and bacteria. Like all eukaryotes it contains a nucleus, surrounded by a nuclear envelope. The envelope consists of two membranes and contains typical nuclear pores (Robinow & Marak, 1966; Matile *et al.*, 1969; Molenaar *et al.*, 1970). On the other hand yeast resembles prokaryotes in that it has a short generation time and no mitotic apparatus.

The DNA content of yeast (about 12×10^9 daltons/haploid genome) is only about ten times that in bacteria (Bicknell & Douglas, 1970).

Compared with other cells, yeasts are very rich in RNA. The amount of RNA is 50–100 times greater than the DNA content. Most of the total RNA of the yeast cell is rRNA (Fukuhara, 1967).

The 80S ribosomes of yeast contain a 19S rRNA species with a molecular weight of about 0.7×10^6 and a 26S rRNA species with a molecular weight of about 1.3×10^6 (Retèl & Planta, 1968; Loening, 1968). In DNA-RNA hybridization experiments about 2% of the yeast genome was found to be complementary