RIBOSOMES

Pedigree and Parturition

from our Molecular Biology Correspondent

THE life cycle of the ribosome, from the ovum to the grave, as it were, is now almost fully documented, and makes one of the more absorbing stories of molecular biology. It begins with the ribosomal genes in the cellular DNA, which are present several times over in the bacterial genome, and hundreds of times in the eukaryotic chromosome. Denaturation maps of eukaryotic ribosomal DNA make this string of identical genes manifest: in this technique the DNA is held at a temperature within its melting range, the melted regions are propped open with formaldehyde and the population of molecules on a grid is examined in the electron microscope. The melted regions, which will have a lower content of (G+C) than those which remain intact, may then be indexed along the chain, when a repeating unit, consisting of (A+T)-rich regions, interspersed with those of high (G+C) in a characteristic pattern, may be seen. Such denaturation maps have been prepared, for instance, for the ribosomal DNA of *Xenopus laevis*. They show a four-peaked profile followed by a featureless tract of rather high (G+C) content. This last corresponds to the "spacer" region, which is eliminated when the 40S precursor RNA is broken down into the 18 and 28S ribosomal subunit RNA species, which comprise the greater part of the ribosomal gene.

Brown, Wensink and Jordan (*J. Mol. Biol.*, **63**, 57; 1972) have now followed up this earlier work by a comparison of the denaturation maps of *X. laevis*, and the closely related *X. mulleri*. The mature ribosomal RNAs of these species are, not surprisingly, identical within the limits of technique in base compositions, as well as physical properties, such as sedimentation and electrophoresis rates and absorbance melting curves. The buoyant densities of the ribosomal DNAs of the two beasts, however, are not identical, and they hybridize poorly with one another. That the difference resides in the spacer segment becomes apparent from the denaturation maps, the *X. mulleri* DNA being much more thermally labile over the greater part of this region, and much more resistant in the lesser. Differences in (G+C) content of different parts of the chains are reflected also in absorbance melting curves of the ribosomal DNA of the two species. It thus seems that although extensive homology between the 28 and 18S RNA sequences persists over a great range of disparate eukaryotes, the spacer regions of the precursor are utterly different between two barely

distinguishable species. This is interesting in itself, but more particularly it raises the basic question of how such a mutation in a multiple gene spreads from one copy to another along the chromosome. Brown *et al.* refer to this as horizontal evolution, and uphold the view that some form of scanning and correcting mechanism must operate.

In an accompanying article, Brown and Blackler (*ibid.*, 75) examine the DNA of hybrids of *X. laevis* and *X. mulleri*—which it seems cannot tell the difference. The essential finding is that the paternal multiple genes turn up in the offspring. The inference is that the transmission of these genes occurs by way of at least one copy from the original chromosomal DNA; it eliminates the alternative that the replication involves an independent episomal body—a mechanism which in principle was on the cards, for the amplified ribosomal DNA is packed into nucleoli at the egg cell stage.

Dawid (*ibid.*, 201) has been concerned with the mitochondrial RNA of *X. laevis*. In respect of nucleic acids the mitochondrion operates an independent ecology from the rest of the cell, and has its own characteristic ribosomes as well as DNA. The molecular weight of its DNA is only some 12 million. Dawid finds that the mitochondrial ribosomes and tRNAs hybridize in all with 20 per cent of one DNA strand, corresponding to one of each ribosomal RNA molecule and about fifteen tRNA molecules per DNA. Thus in mitochondria there is—uniquely it seems—no gene amplification. (The curious dearth of tRNA species—apparently not even one per amino-acid, let alone the usual degenerate set of sixty or so—is unexplained. Perhaps the balance are imported from the cytoplasm.)

In a second article, Dawid and Chase (*ibid.*, 217) grapple with the problem of molecular weight determination of the mitochondrial RNA. The ribosomes have been reported to be much smaller than those of bacteria—to which mitochondria are generally likened—and it would not therefore be surprising if the ribosomal RNA were not also small. Melting curves suggest a low degree of pairing, and hence perhaps a relatively expanded structure. This being so, physical measurements, such as sedimentation and electrophoresis on the native molecule, provide, Dawid and Chase assert, a poor estimate of the molecular weight, on the grounds that the species used for calibration are of a different character. By setting up new calibrations in solvents in which base pairing is annihilated, they arrive at molecular weight values which are indeed very low—little more than half of those of the

corresponding *Escherichia coli* species. This article deserves attention outside the field with which it is concerned, for its forthright attempt to meet the perpetual torment of molecular weight determination. Whether it represents the last word (it neglects, for example, to consider variations in partial specific volume) is not yet clear.

On the subject of ribosomal precursors, Kumar and Warner (*ibid.*, 233) have analysed the proteins of the nucleolar particles of HeLa cells, and have found a series of proteins that are not present in the matured ribosomes. Their labelling patterns show them to be long lived, and evidently to remain in the nucleolus after the true ribosomal proteins are expelled, so that they are probably re-used. They are surmised to have some function in the maturation process. Wengler, Wengler and Scherrer (*Europ. J. Biochem.*, **24**, 477; 1972) report that ribonucleoprotein particles, which are of mitochondrial origin, by the criterion of repression by ethidium bromide and not by actinomycin D, turn up in the postmitochondrial cytoplasmic fraction. They suggest that contamination with these species may have misled workers interested in messenger-containing particles emerging from the nucleus. Mirault and Scherrer (*FEBS Lett.*, **20**, 233; 1972) have found a nuclease in the nucleolus of HeLa cells, which is able to break down the 45S nucleolar ribosomal precursor into pieces containing 28 and 18S RNA, as well as some intermediate states. No nucleotides are released, and the enzyme is therefore evidently an endonuclease, which nips the chain in only a few places. It is hoped that this may be a true maturation enzyme.

NEONATES

Lecithin and Respiration

from a Correspondent

ALTHOUGH the mortality rate for premature infants in the first few weeks of life has progressively fallen with greater care and knowledge in their management, many still die of respiratory distress syndrome (RDS)—a disorder in which the lungs do not function normally. Advances in the treatment of RDS are being made but it would obviously be preferable to find ways of preventing its occurrence.

The disorder seems to be due to lack of a surface-active substance produced by cells lining the lungs without which the lungs do not fully expand when the infant is born. Lecithin is thought to be responsible for this surfactant activity and it is known that the lecithin present in the lungs of infants dying of RDS is much less than in those who die from other causes. *In utero* the fluid within the lungs mixes with the