Craven and Gupta report analogous results when the 30S ribosomes are treated with iodoacetate or 2-methoxy-5-nitrotropone (derivatizing —SH and free $—NH_2$ functions respectively). They have also used trypsin insolubilized by attachment to a cellulose matrix, as an agent exclusively restricted to acting at the surface of the particles. This method showed that certain proteins are not attacked by any of these agents, whereas others are consistently accessible to the action of all three. A further class displays intermediate reactivity towards these agents. Once again the accessibility of these proteins is inversely related to their positions in the assembly map.

It is concluded therefore that the proteins which bind to the 16S RNA early in the reconstitution are sheltered within the interior of the 30S ribosomal particle, with the late-binding proteins predominantly towards the outside. This result is a further contradiction of the models of ribosomal structure previously proposed, in which the proteins would be more or less equally accessible to the action of enzymes or chemical reagents, and it reaffirms that the order of assemblage is in part determined by protein–protein interactions.

NUCLEOLI

rRNA Synthesis in *Urechis*

from a Correspondent

THE activities of the fibriller core (which presumably contains the DNA coding for rRNA) and granular cortex of the nucleolus have been extensively and successfully pursued in a wide range of organisms. Cytological studies suggest that RNA is first synthesized in the core and it then moves to the cortex. Biochemical analysis shows that this RNA is the precursor to ribosomal RNA and that this is cleaved and processed to the mature ribosomal particles in the nucleolus. The use of biochemical and cytological techniques in one system has enabled Das *et al.* (*Proc. US Nat. Acad. Sci.*, **67**, 968; 1970) to correlate the labelling of the pre-rRNA components in eggs of *Urechis caupa* with autoradiographs of the nucleoli. The eggs of this marine worm are easily obtained and are free of the follicle cells which interfere with biochemical studies in amphibian eggs. Their slow rate of processing of precursor rRNA makes it easier to follow the fates of all the components. The one very large nucleolus gives good autoradiographs and the rDNA is easily detected in the cores by Feulgen staining. And most important, clean nucleoli can be isolated from the eggs without degradation of the pre-rRNA.

After labelling for 1 to 2 h with ^{3}H-uridine, Das *et al.* found radioactivity in the 38S precursor rRNA (by sucrose gradient centrifugation); silver grains were confined to the nucleolar core. After several hours, the cleaved products of the 38S precursor, the 30S and perhaps 20S precursors to mature rRNA, were labelled, and radioactivity had spread to the cortex. Das *et al.* used actinomycin D to inhibit further RNA synthesis after 2 h labelling and incubation continued for a further 6 h. Then only the 30S and 20S components were labelled and silver grains were confined to the granular cortex. The 38S, 30S and 20S RNA thus methylated after incubation in methyl-labelled methionine. This work probably confirms that the components are ribosomal, but it might be useful to take the experiments further to investigate the processing of the pre-rRNA.

What Das *et al.* did not mention is that the *Urechis* oocyte represents a special case of ribosome synthesis, in which the large size of the nucleolar core seems to be caused by an increased number of ribosomal genes. Dawid and Brown (*Dev. Biol.*, **22**, 1; 1970) have examined the amplification of mitochondrial DNA and of rDNA and have shown that the nucleus of *Urechis* oocytes contains about six times the normal complement of ribosomal genes. Their conclusion is based on hybridization of *Urechis* DNA with *Xenopus* rRNA in non-saturating amounts—not the best of conditions, but their argument seems reasonable. They have shown that *Xenopus* rRNA is about 60 per cent homologous with the rDNA of *Urechis*, fractionated on a caesium chloride gradient, and that the amount of RNA hybridized is proportional to the amount of DNA immobilized on the filters. *Urechis* oocyte DNA hybridized with about three times as much RNA as sperm DNA. Because the oocyte also contains a large excess of mitochondrial DNA, about 60 per cent of the total per cell, it seems that the nuclear DNA contains rDNA amplified at least six-fold.

These results may be compared with those in Amphibia and water beetles, as studied in Birnstiel's and Gall's laboratories. The mature *Urechis* egg contains, according to Dawid and Brown, 12 ng of RNA which is about 3×10^9 ribosomes, some 1,000 times more than in somatic cells. But the rDNA is amplified only six-fold and in one nucleolus, which may be contrasted with the 1,000 or more extrachromosomal nucleoli, in *Xenopus* oocytes. In both cases sufficient maternal ribosomes are made for early embryonic development, and rRNA synthesis does not need to start again until later in embryogenesis. Thus, amplification of rDNA seems to be associated with extensive synthesis and storage of ribosomes for later use, whether this amplification is in one nucleolus or many. This correlation suggests that the extra chromosomal rDNA may be under different control from the rDNA integrated in the chromosomes of somatic cells. Such a difference would permit oocytes to uncouple the synthesis of ribosomes from protein synthesis, in distinction to somatic cells where these are tightly coupled.

ENZYMES

Spectroscopist's Guide

from our Molecular Biology Correspondent

MANY a sanguine hope has been expressed about the promise of various spectroscopic techniques for determining stereochemical details of interactions at active sites of enzymes, and observing catalytic events. As long as X-ray crystallography remains a static method, they are obviously worth nurturing, even if so far few very memorable results have accrued. Some interesting explorations, using fluorescence, electron spin resonance and nuclear magnetic resonance are to be found in the November issue of the *Proceedings of the National Academy of Sciences*.