RIBOSOMES

Picking up the Pieces

from our Molecular Biology Correspondent

In many a laboratory, especially since the impact of Nomura's work on stepwise reassembly has made itself felt, ribosomes have been scourged with salts, tanned with aldehydes and devoured with nucleases in attempts to extract from them fragments of RNA still associated with proteins. By some way the most encouraging and informative study so far is that of Allet and Spahr, which has just appeared in the *European Journal of Biochemistry* (**19**, 200; 1971). This is a polished piece of work, which gives rise to some thought provoking conclusions: treatment of 50S subunits of *Escherichia coli* with pancreatic ribonuclease under mild conditions introducing a single break in the RNA chain. This becomes apparent when the RNA is extracted: gel electrophoresis shows the progressive conversion of the 23S chain into two fragments, which differ in their oligonucleotide maps, and only one of which contains the 3′ terminal sequence. The break in fact occurs two-fifths of the way along the chain from the 5′ end.

The two fragments evidently remain cemented together by proteins, but when the salt-labile "split proteins" are dissociated by treatment with lithium chloride, they separate and can be collected from a sucrose gradient. When the components of the two pieces were separately analysed, a startling discovery came to light: only two proteins were unique to each fragment. These, one would be tempted to infer from Nomura's results on the 30S subunit, may be the primary binding species, which associate with the RNA and direct the uptake of further proteins, and the correct folding of the particle. The remaining fifteen or so "core" proteins are distributed in varying but reproducible proportions, between the two fragments. To define the specificity of the interaction more explicitly, Allet and Spahr then examined the binding of labelled "split proteins" with the separated core particles. Both core particles are able to bind significant amounts of the various split proteins, though some of these show a preference for one or the other. When the core particles are mixed, however, there is a large increase in the extent of binding of many of the split proteins, and the 50S subunit is in effect regenerated. Such cooperativity of protein uptake was of course described by Nomura and his colleagues in the assembly of the 30S subunits from the vestigial particle, containing only a few primary structural proteins. On reflexion therefore the results of Allet and Spahr can be seen to make good sense. The structure of the 50S subunit is evidently maintained by a network of binding sites on the proteins, and when the RNA is cleaved, some species remain anchored at one end, some at the other. The primary binding proteins, which direct the reassembly, bind non-cooperatively and uniquely to one or other fragment of the RNA.

A more rudimentary ribonucleoprotein particle can be obtained from eukaryotic ribosomes by treatment with EDTA, and contains messenger, rather than ribosomal, RNA. Thus far such particles, and their proteins, have been poorly characterized, an omission which has now been rectified to a noteworthy extent by a study from Chantrenne's laboratory (Lebleu *et al.*, *ibid.*, 264). In the first place, under more stringent conditions of sedimentation a contaminating particle is resolved. This sediments at 8S, compared with 15S for the messenger complex, and contains 5S ribosomal RNA and a protein of 45,000 molecular weight—presumably a ribosomal protein. The 15S complex, freed of its impurity, contains only the messenger, and two proteins of high molecular weight, one of them especially at 130,000 (estimated by detergent-gel electrophoresis) much larger than anything normally characterized as a ribosomal constituent. The 15S particle associates strongly with 40S subunits, and the isolated messenger also binds to an appreciable extent. If, however, the subunits are first washed with deoxycholate, the messenger fails to interact, whereas the messenger-protein complex binds with undiminished efficiency. Conversely, polysomes washed with deoxycholate before treatment with EDTA release free messenger instead of the 15S particle. The proteins in the 15S particle are evidently "factors", vital in some way for utilization of the messenger.

An interesting paper by Herzog, Ghysen and Bollen (*Mol. Gen. Genet.*, **110**, 211; 1971) concerns the "dissociation factor" in *E. coli*, which is thought to be responsible for dissociating the 70S ribosomes into subunits after their traverse of the messenger, in preparation for re-initiation. In a mutant strain of cells, dissociation of monosomes by lowering the magnesium concentration is distinctly inhibited. The ribosomal protein pattern is identical with that of the wild type, and it emerges that it is the dissociation factor, which can be prepared by a salt wash, that is defective, when tested on wild type ribosomes. The effect vanishes when an amber suppressor is present, and is therefore an amber mutation.

Electromagnetic Noise generated in the Ionosphere

In the forthcoming issue of *Nature Physical Science*, D. G. Cartwright and P. J. Kellogg of the University of Minnesota discuss the results of a new rocket-borne experiment carried out in the ionosphere. They have detected electromagnetic radiation associated with an electron beam, of controlled flux and energy, injected into the magnetosphere from the rocket.

The background to the experiment is described by R. A. Hendrickson, R. W. McEntire and J. R. Winckler, also at Minnesota, in this issue of *Nature* (page 564). In the experiment, more than 3,000 short pulses of electrons, of 35 to 45 keV energy and at such pitch angles that they become trapped by the geomagnetic field, were injected at altitudes above 150 km. These electrons were observed to bounce between the mirror point near the rocket and that at the conjugate point, and back, in 0.62 s. As the electrons also drifted eastwards on a constant L shell ($L \sim 2.5$), they were observed by detectors aboard the rocket which was launched eastwards from Wallops Island, Virginia.

The electric field of electromagnetic radiation within the frequency range 16 Hz to 10 MHz, generated by the electron beam, was measured by equipment mounted on the ejected fibreglass nose cone of the rocket. While the electron gun was firing, radiation was observed at frequencies $\sim$5 MHz, between the local plasma frequency and the upper hybrid resonance frequency, and also at frequencies $<$1MHz. Čerenkov emission from the beam of electrons is thought to be responsible for the radiation, which propagates at low phase velocities in the extraordinary and whistler modes respectively. Radiation observed at $\sim$2 MHz, less than twice the electron gyro-frequency, is consistent with the first Bernstein mode in a warm plasma; the particle-wave interaction producing this radiation is not understood. A spin modulated band of noise at $\sim$8 kHz is interpreted as being noise at the lower hybrid resonance frequency caused by argon ions which were emitted to keep the payload electrically neutral while the electron gun was firing. Čerenkov radiation is again the favoured emission process with propagation taking place in the whistler mode.

The important conclusion of this work is that the electron beam loses less than only 1 millionth of its energy to plasma instabilities or to wave emission. Cartwright and Kellogg do not, however, understand all the phenomena observed; marked changes in the characteristics of the signals are observed part way through the flight at the time of the (rather sinister) "black cloud".