SELFASSEMBLY

Tricks with Ribosomes

from our Molecular Biology Correspondent

SINCE Nomura and his colleagues achieved the small miracle of mixing 16S RNA with its complement of some twenty ribosomal proteins to give active *Escherichia coli* 30S subunits, strenuous and hitherto unsuccessful attempts to reassemble the large subunit of the same species have been made in many laboratories. True, Nomura's group succeeded with 50S ribosomes from the thermophilic organism, *Bacillus stearothermophilus*, so that the principle has been in no doubt. Now, however, Maruta, Tsuchiya and Mizuno (*J. Mol. Biol.*, **61**, 123; 1971) have found the conditions for making active *E. coli* 50S subunits from their constituents, and more, regenerating the small and large subunit simultaneously in a single mixture of the three ribosomal RNA species and all the proteins—a process which may be compared to assembling a watch by putting all the parts in a bag and shaking.

The trick, so far as the 50S subunit is concerned, is to avoid exposure of the proteins to the solvents that are normally used for extraction and dispersion. Instead they are freed of RNA by treatment with nuclease. The native ribosomes are very resistant towards nucleases, but after partial unfolding in a medium containing molar ammonium chloride, the RNA becomes labile. When treated with the endogenous *E. coli* ribonuclease II, therefore, the RNA is hydrolysed to oligonucleotides, and the proteins are left, mostly as a precipitate, which will dissolve in a concentrated buffer solution. This is the protein preparation used in the reconstitution experiments, for if any attempt is made to remove nucleotide material by dialysis, no ribosomes can be regenerated. Evidently, therefore, some of the 50S ribosomal proteins are easily converted into a state from which specific interaction with the other ribosomal constituents becomes impossible. Bovine serum albumin is also included in the mixture as an obscurely beneficial component.

The reassembly reaction has a number of remarkable features. As with *B. stearothermophilus* ribosomes, the functional 50S subunit is not formed unless 30S subunits are present, or alternatively, as Maruta *et al.* now find, the mixture of the proteins and RNA of the 30S subunit. Moreover when only the proteins originating from either the 50S or 30S particle are labelled, all the label reappears in the right place after reassembly. That is to say none of the proteins from either subunit are captured by the other subunit, in spite of their basicity and the stupefying complexity of the system. The resulting

ribosomes are active in poly U and viral RNA-dependent polypeptide synthesis. The 5S RNA which resides in the large subunit is indispensable for activity. It may be added separately, as a final step in the reconstitution process.

This last effect—the reversible release of 5S RNA from the 50S subunits—has been studied in other laboratories. Gormly, Yang and Horowitz (*Biochim. Biophys. Acta*, **247**, 80; 1971) have also used concentrated ammonium chloride at low magnesium concentrations to bring about a partial relaxation of the subunit to a more slowly sedimenting state. Liberated with the 5S RNA under these conditions are several ribosomal proteins, four species being completely and another three partially dissociated. On returning the ribosomes to their usual magnesium concentration, the 5S RNA is taken up, and folded subunits again appear. This process, however, also required the pre-

sence of the dissociated protein fraction—a conclusion apparently at odds with a report last year by Reynier and Monier. Unfortunately the restored ribosomes appeared not to be biologically active.

Gray and Monier (*FEBS Lett.*, **18**, 145; 1971) have tried another approach to identifying the ribosomal proteins directly associated with 5S RNA. They incubate 23S and 5S RNA with fractions of 50S ribosomal proteins and detect the formation of complexes by the membrane filtration method. A magnesium-dependent stable complex is indeed formed with one column fraction of 50S proteins. This complex can be isolated by sucrose gradient sedimentation, and when the proteins are analysed by gel electrophoresis three major and two minor zones can be resolved. One of the former is associated with 23S RNA even in the absence of 5S.

Infrared and the Black-body Background

SINCE the discovery by Penzias and Wilson (*Astrophys. J.*, **142**, 419; 1965) of 7 cm radio noise, thought to be remnant cosmological radiation with a black-body temperature of about 2.7 K, much attention has been paid to measuring the background radiation of the sky at wavelengths from the near infrared out to the radio region. Measurements which verify the possible existence of the background radiation have been made on the long wavelength side of the black-body peak, predicted to be at about 1.5 mm, but observations near the peak and at shorter wavelengths are not possible from the ground because of atmospheric absorption.

In recent years rockets have been used for investigating the sky background in the millimetre, sub-millimetre and infrared spectral regions from above the atmosphere. Two contributions on this topic appear in next Monday's *Nature Physical Science* and present conflicting measurements of the far-infrared background levels. D. P. McNutt, K. Shivanandan and P. D. Feldman report results of a recent Naval Research Laboratory flight in which they measured sky background in the 10 to 30 μm , 100 μm and 280 μm regions. A. G. Blair reports on rocket measurements obtained by the Los Alamos group in the 65 to 70 μm region.

The NRL experiment was launched on a Black Brandt VB rocket from Wallops Island on February 24, 1971, at 2013 EST and data were obtained between altitudes of 130 km and 275 km over a time period of 135 s. The photometer was cooled to a temperature of 2.8 K and the intensity calibration was estimated to be accurate to within a factor of two. Results indicated that at zenith angles less than 20°, scattered

earthshine was unimportant, and the signal levels were interpreted in terms of a true background 100 μm radiance of $1.4 \times 10^{-9} \text{ W cm}^{-2} \text{ sr}^{-1}$ and a 280 μm radiance of $2.9 \times 10^{-9} \text{ W cm}^{-2} \text{ sr}^{-1}$.

The Los Alamos experiment was launched from Kauai Test Range on May 29, 1971, at 0048 HST and carried a radiometer cooled with liquid helium to measure background fluxes in the spectral region 65 to 170 μm and 0.8 to 6.0 mm. The 0.8 to 6.0 mm results are not inconsistent with the proposed background 2.7 K black-body cosmic radiation. These results are discussed in a previous publication (*Phys. Rev. Lett.*, **27**, 1154; 1971). In the 65 to 170 μm region, Blair puts an upper limit of $1.2 \times 10^{-10} \text{ W cm}^{-2} \text{ sr}^{-1}$ on the 100 μm radiance. This figure is consistent with previous results obtained by both the NRL group (*Science*, **167**, 1277; 1970) and the Cornell group (*Nature*, **281**, 375; 1971) but is more than an order of magnitude less than the current results of McNutt *et al.*

In looking for reasons to explain this large difference, McNutt *et al.* discard the possibility that they are detecting ionospheric radiation. Summarizing all rocket data available for the 100 μm and 280 μm regions, they conclude that those flights near local midnight show comparable low radiance levels whereas the earlier Cornell experiment (*Science*, **164**, 1271; 1969) and their current results—both recorded near astronomical twilight—are significantly higher. They suggest the possibility of a contribution from the outer magnetosphere, or near-Earth interplanetary space, which could be subject to a diurnal variability. They reject, however, the likelihood of a correlation between radiance and geomagnetic index K_p .