three months, but most will stay for six months or more. Already about a dozen chemists and physicists—from Italy, France, Holland, Israel, Great Britain, the United States and the Soviet Union—are planning to be there when the centre opens in six or nine months' time. The more senior will continue their own work and the more junior will either cooperate with them or pursue their own ideas.

RIBOSOMES

Putting Them Together

from our Molecular Biology Correspondent

MUCH the most complex system that has been made to assemble spontaneously from its components *in vitro* is the 30S *E. coli* ribosome. The components are a long, flexible coil of RNA and some twenty proteins, mostly basic, and therefore possessed of a high random affinity for the RNA so that a quite extraordinary degree of precision must be involved in the recognition mechanisms operating in the system. From the quite general viewpoint of specific interactions between biological molecules, the latest study by Traub and Nomura (*J. Mol. Biol.*, **40**, 39; 1969) is of the widest relevance.

They report in the first place the environmental requirements for the reassembly to proceed. The ionic strength is especially critical, and with potassium chloride as the primary electrolyte, there is a very sharp optimum at 0.37. This must be supposed to represent a balance between strong non-specific polyacid-polybase interactions expected at low ionic strengths, which would destroy the discrimination against incorrect associations, and the extensive weakening of interactions when salt concentration is high. The temperature is also critical: between about 25° and 40° C there is a sharp rise in the extent of reconstitution, no appreciable activity being recovered at low temperatures. There is also an absolute requirement for magnesium ions at a concentration higher than 10^{-3} M. The pH optimum is broad, because appreciable changes in ionization of groups on the protein and RNA do not occur between about 6 and 8.

The most striking feature of the process is its close adherence to first-order kinetics, in spite of the number of components involved, and indeed it is unaffected by the total reactant concentration. Traub and Nomura infer that there is a rate-determining step, involving possibly a conformational rearrangement of a complex, and they have gone a long way towards confirming this hypothesis. When the RNA and the proteins are mixed at 0° C, only partial combination occurs, and electrophoretic analysis of the supernatant after centrifuging out the nucleoprotein complex shows that a group of proteins remains, which is identified with the components released from native 30S ribosomes on treatment with concentrated caesium chloride solutions (the *S*-proteins). When, however, the intermediate nucleoprotein particles—which are essentially unfolded, sedimenting at about 21S—are warmed to 40° C, they acquire the capacity to take up the group of *S*-proteins, with formation of active 30S ribosomes. It seems highly likely then that the rate-determining step consists of activation of the first RNA-protein complex, which is indeed found to be a first-order process with rate and activation parameters identical

to those characterizing the overall reassembly reaction. The *S*-proteins do not interact with free RNA, and presumably associate with the other ribosomal proteins *in situ*. Traub and Nomura allude to forthcoming evidence that the rearrangement is cooperative, and that only two or three binding sites on the RNA are involved in the reaction.

As a satisfactory by-product of this work, Traub and Nomura have also solved the problem of how to regenerate functional particles from subunits unfolded by exposure to EDTA to extract the magnesium. This treatment leads to slowly sedimenting entities, which earlier workers had altogether failed to reconvert into folded functional subunits. The key is the temperature-dependent rearrangement: warming to 40° C regenerates active 30S ribosomes. Moreover, it seems that loss of the *S*-proteins is involved, and that these recombine only after the internal rearrangement step.

Traub and Nomura comment finally on the relation between their results and the assembly of ribosomes during biosynthesis: the generation time of *E. coli* shows that *in vivo* the assembly must be considerably faster. Whether the medium composition or other factors are responsible can only be guessed at. It is suggested, however, that the same principles operate *in vivo* as *in vitro*, and indeed the well known existence of precursor particles of low sedimentation coefficient offers good grounds for speculation.

MITOCHONDRIA

Were They Bacteria?

from our Cell Biology Correspondent

REPORTS of experiments supporting the notion that mitochondria may have evolved from endosymbiotic bacteria continue to appear. Last December, Smith and Marcker (*J. Mol. Biol.*, **38**, 241; 1968) described a particularly compelling experiment. N-formyl-methionine *t*RNA ($tRNA_{F}^{met}$), the *t*RNA required to initiate protein synthesis in bacteria, has not been identified in eukaryotes, except in algae such as *Euglena gracilis*, where it occurs in chloroplasts, and in yeast. But, contrary to expectation, there is less $tRNA_{F}^{met}$ in yeast cells than in bacteria; if this species of *t*RNA was required for the initiation of yeast cytoplasmic protein synthesis yeast cells should contain more of it than do the smaller *E. coli* cells. A possible explanation for this discrepancy was that the $tRNA_{F}^{met}$ in yeast was confined to the mitochondria, and Smith and Marcker convincingly showed that this is the case. Initiation of protein synthesis in yeast mitochondria is similar to that in bacteria; on the other hand, they found no trace of $tRNA_{F}^{met}$ in yeast cytoplasm.

Küntzel, working with another fungus, *Neurospora crassa* (*Nature*, **222**, 142; 1969), reports that the proteins from both the large and small subunits of *Neurospora* cytoplasmic and mitochondrial ribosomes are almost totally unrelated, as judged by their behaviour on carboxymethyl cellulose columns.

But, in spite of this difference in the species of proteins in the two classes of ribosomes, it seems that the mitochondrial ribosome proteins are actually synthesized in the cytoplasm on cytoplasmic ribosomes. The evidence for this comes from experiments with inhibitors of bacterial and mitochondrial protein synthesis which have no inhibitory effect on cytoplasmic protein