Self Assembly of *R17*

from our Cell Biology Correspondent

EARLIER this year (see *Nature*, **214**, 1074; 1967) Sugiyama *et al.* reported that incubating *MS*2 phage coat protein with *MS*2 RNA produces phage-like particles. Although these self assembled particles cannot be distinguished from authentic phage in the electron microscope, they are not infectious and sediment at 70*S* instead of 80*S*. Since then Hohn (*Europ. J. Biochem.*, **2**, 152; 1967) has done similar experiments with the closely related phage *fr* and obtained essentially identical results. These non-infectious particles, assembled *in vitro*, closely resemble defective non-infectious particles produced *in vivo* during replication of certain amber mutants (A cistron or Sul mutants) of *fr* and the closely related phage f_2 and *R*17. Apparently, both types of non-infectious particle are defective because they lack a minor protein component, the A protein specified by the A cistron, which is present in intact infectious RNA phage (Nathans *et al.*, 1966; Argetsinger Steitz, 1967) and somehow ensures either the correct encapsulation of the phage RNA or the absorption of the phage to *E. coli* hosts or both.

The obvious experiment was to add this missing A protein to the coat protein/phage RNA mixture and see if infectious phage are reconstituted. The great difficulty, of course, was isolating enough A protein; each intact phage probably contains only one molecule of A protein and this has to be separated from the 180 coat protein molecules. Argetsinger Steitz has done this; the A protein apparently has a molecular weight of about 35,000, and although it is highly insoluble in aqueous buffers enough has been obtained to do the self assembly experiment, and Roberts and Argetsinger Steitz now report the successful *in vitro* assembly of infectious *R*17 phage (*Proc. US Nat. Acad. Sci.*, **58**, 1416; 1967).

When A protein is added and dialysed with a mixture of *R*17 coat protein and RNA, the yield of infectious phage increases by a factor of several hundred. The titres of infectious phage obtained are impressively high, up to $1{\cdot}3 \times 10^7$ phage/ml., although the efficiency of reconstitution is only 2×10^{-6}. The maximum yield of infectious phage is obtained when one A protein molecule per phage RNA molecule is added; more A protein does not increase the yield. Two factors probably account for the low efficiency of assembly of infectious phage. First, RNase almost certainly contaminates the isolated components, and this would degrade the *R*17 RNA before it is incorporated into particles. Second, the protein and RNA must necessarily be subjected to harsh treatments during isolation and are probably partially inactivated as a result. When the complete assembly mixture was analysed on a sucrose gradient, the bulk of the reconstituted particles were 70*S* defectives, but the majority of the infectious particles sedimented at 80*S* like wild-type phage. Mild RNase treatment of the reaction mixture completely removed the 70*S* defectives leaving only the 80*S* infectious particles, but even some of the infectious particles are sensitive to RNase whereas normal phage is completely resistant. Clearly some of the reconstituted phage, though infectious, are not quite normal: it seems probable that these arise because some of the protein incorporated is partially damaged and does not protect the RNA from degradation by RNase.

Despite these qualifications, this experiment is the first in which a phage has been reconstituted *in vitro*, albeit at low efficiency, from two species of protein and a nucleic acid, and this has considerable implications for ideas of organelle self assembly. Furthermore, the experiment provides definite proof that the A protein is a necessary constituent of infectious RNA.

No Life on Life Origins

from our Special Correspondent

THE first public meeting on November 2 at the opulent new premises of the Royal Society was a disappointing affair. As Sir Robert Robinson said when introducing the morning session, the subject, "Anomalous Aspects of Biochemistry of Possible Significance in Discussing the Origins of and Distribution of Life", was particularly fitting for the occasion because it provided an opportunity for the sort of speculation and intellectual curiosity which had excited the founders of the Royal Society. In the event, the speakers and the audience rarely responded to this challenge. Instead, there was a series of thirty minute reviews—inevitably superficial for lack of time—of the occurrence in some biological systems of carbon, halogen, silicon and vanadium compounds and of why ATP is the universal mediation of biosynthesis. But speculation about either the terrestrial origin of life or the possibilities of extraterrestrial life was really not noticeable. The speakers, many of whom no doubt seldom view their work in this light, may have done their best, but the general discussion never came to life and was very much restricted to the here and now. The smell of the lunch being cooked in the kitchens below was, perhaps, too distracting.

In the afternoon, Professor H. E. Hinton (University of Bristol) described his experiments on suspended animation in insects which prove that living systems can survive alternate cycles of hydration and dehydration—some species apparently survive exposure to temperatures varying from below the boiling point of helium to +104° C after total dehydration. He then gave several cogent reasons for believing that life originated on the surface of the Earth rather than in a continuously wet environment, the chief one being that only in niches of rocks or dust particles, or in small pools subjected to drying, would concentrations of chemicals have become sufficiently high for macromolecules to be formed. Dr M. V. Tracey (New South Wales) then described fascinating experiments on the way stimulants of the central nervous system and anaesthetics induce ordered clusters of water molecules in bread dough, of all unlikely things. He suggested that plant alkaloids originated because of their ability to regulate cell water content and postulated a closed circulation plant. Then there was a description of an anaerobic ecosystem based on the sulphate/sulphide transformations and the possibility that such systems could well be similar to an early stage in the evolution of life after its origin. The last paper reviewed the great diversity of compromising environments which support microbial life, including a brief mention of experiments with simulated Martian conditions. The applause woke the sleepers, and after a few questions the meeting closed.