Genetics and Structure of Virus Genomes

from a Correspondent

THE Virus Group of the Society for General Microbiology, in contriving to satisfy the interests of the diverse breeds of virologists, generally succeeds in arranging meetings with a very varied menu. The symposium and open papers arranged by the group in Glasgow on July 5 and 6 were no exception and those present were regaled with tales of wilting pea plants, with complex analyses of oligonucleotides, with slides of dehydrated mosquitoes and assassin bugs, as well as with the inevitable profiles of polyacrylamide gel electropherograms.

The chief symposium was on the genetic and structural aspects of virus genomes and, after a very lucid and personalized introduction to possible mechanisms of genetic recombination by Professor N. D. Symonds (University of Sussex), the next three contributions were, in effect, progress reports from Glasgow's Institute of Virology. Drs J. F. Williams and C. S. H. Young described their attempts to use genetical techniques to unravel the intricacies of adenoviruses. Using type 5 temperature sensitive mutants they can now show seventeen complementation groups—a very considerable feat considering the relative difficulties in plaquing adenoviruses. Recombination frequencies verging from 0.1 to 15% have been obtained with these mutants and a preliminary linkage map using two factor crosses has been derived. A third marker involving heat stability, which has been recently defined, should prove useful in substantiating this map.

The Glasgow group has also been able to carry out experiments analogous to those used by Snustad with DNA phages and accordingly has been able to assign tentatively stoichiometric and catalytic functions to the appropriate genes. Similarities to the pattern of results obtained by phage workers was also the final conclusion of Dr D. A. Ritchie's contribution. He described some preliminary results obtained using herpesvirus type 1 temperature sensitive mutants and a syncytial plaque marker in examining reciprocity in recombination. Dr C. R. Pringle, the last of Glasgow's trio, in reviewing the genetics of animal RNA viruses, described some recent results in which he and his colleagues were able to grow vesicular stomatitis (VSV) and respiratory syncytial viruses (but not pseudorabies virus) in cells devoid of nuclei, using a technique developed by E. A. C. Follet. Temperature sensitive mutants of VSV are being analysed in some detail with respect to both transcription and translation and the results suggest, as with the RNA phages, that the two processes may be intimately coupled.

Dr M. Pons (Public Health Research Institute, New York) completed consideration of animal viruses by reviewing some aspects of the structure of influenza virus ribonucleoprotein and provoked a lively challenge from the floor in relation to his interpretation of results with short labelling periods. A later shorter contribution, in which he suggested that most of the recombination with influenza viruses could be attributed to the clumping of virus particles, similarly stimulated some verbal interplay. The interesting group of plant viruses which have divided genomes were competently reviewed by Dr A. van Kammen (State Agricultural University, Wageningen). He described mutants of cowpea mosaic virus which allowed the relevant phenotype to be ascribed to a particular population of viruses, and because this virus can now be grown in protoplast cultures there should soon be some better understanding of the molecular mechanisms involved.

To complete the picture, the animal virologists were shown the way ahead by two phage-oriented contributions. Dr J. Hindley (University of Bristol) discussed current knowledge of the biochemistry of RNA phages, and Dr K. Murray (University of Edinburgh) described his eventually successful attempts to sequence the sites of restriction enzyme binding. The results with the restriction enzymes and with other similar binding proteins indicated the importance of rotational symmetry in the bases at the critical sites.

NUCLEIC ACIDS

To Catch a Gene

from our Molecular Genetics Correspondent

THE success of experiments with *Escherichia coli* on the control of transcription of genes into messengers has in large part been made possible by the ability to isolate specific genes—or at least small pieces of DNA containing them—in the test-tube ; and the advances in haemoglobin synthesis which have been made since reverse transcriptase was applied to globin mRNA to make a synthetic gene sequence emphasize the advances that might follow the synthesis or isolation at will of any eukaryotic gene. "A general method of gene isolation" is the title of an article by Shih and Martin (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1697 ; 1973) which reports a method for RNA-DNA hybridization able to isolate the sequences of DNA complementary to any purified RNA.

An RNA preparation is chemically linked to cellulose by a water soluble carbodiimide ; this preparation forms a reassociation column, maintained at 37° C in 50% formamide and a saline citrate solution, in which single strands of DNA anneal to the immobilized RNA. One problem in hybridization experiments in a closed incubation mixture is that renaturation of the single strands of DNA prevents them from annealing to RNA. Shih and Martin therefore linked their reassociation column to a denaturation column, filled with glass beads or wool to reduce the circulation time, and maintained at 90° C in the same formamide-salt solution. A pump is responsible for circulating DNA fragments, in 50% formamide–salt citrate, around the system ; DNA binding to RNA remains in the reassociation column but any DNA failing to anneal is pumped through the denaturation column, which ensures that it is single stranded before it recirculates.

The system was tested by binding SV40 RNA to the cellulose of the reassociation column and providing a circulating preparation of SV40 DNA mixed with *E. coli* DNA. The SV40 DNA is specifically retained on the cellulose column even in the presence of a 10,000-fold excess of the *E. coli* DNA, almost all of which is recovered as part of the recirculating system. Specific hybridization of purified RNA with DNA has of course been achieved before. The importance of this new technique is that it allows the hybrid to be exhaustively formed by recirculation and then to be isolated in quantity. In principle, it should be possible to isolate the DNA corresponding to any gene whose RNA can be purified. This may prove to allow the isolation of specific genes from mammalian DNAs, in particular the sequences corresponding to the genomes of the tumour viruses.