category as a "conflicting" assignment.

All but eight of the chromosomes other than no. 1 have genes assigned to them, eighteen confirmed and the rest provisional. Dr D. Bootsma (Erasmus University, Rotterdam), speaking for the committee that pondered this subject, reported that these include five loci for the production of 5S RNA (on chromosomes 13, 14, 15, 21 and 22), which have been identified by *in situ* hybridization. By this technique labelled DNA or RNA can be annealed to intact chromosomes which have been treated with DNA denaturation agents. About the genes provisionally assigned to these chromosomes there remains much disagreement and disbelief. The gene for HLA antigen, for example, was recorded as residing on chromosome no. 6, but the general feeling seemed to be that it would be moved very soon.

There is apparently even more difficulty in deciding on the order of genes on the chromosomes. This aspect of mapping gave considerable trouble to the committee charged with reviewing the genetic constitution of the X chromosome. So far this chromosome has well established loci for four enzymes. Their order on the chromosome is thought to be phosphoglycerate kinase (PGK), α-galactosidase (α-Gal), hypoxanthine-guanine phosphoribosyltransferase (HGPRT), glucose-6-phosphate dehydrogenase (G6PD). But, as the committee's spokesman, Dr P. S. Gerald (Children's Hospital, Boston), pointed out, there is still much disagreement about details.

As so much remains to be agreed and discovered about the human chromosome map, it was hardly surprising that part of the workshop was devoted to such mundane problems as nomenclature, methodology and the availability of experimental material. The discussion of nomenclature proved long and complex, and to tackle the problem further the participants agreed to the establishment of two standing committees to make recommendations for (1) chromosomes and (2) phenotypes and gene symbols. Presumably the committees will present their conclusions when the second workshop convenes next year in Rotterdam.

To make useful cell material available to the genetic community, participants were urged to send samples of their own cell lines to the mammalian genetic mutant cell repository at the Institute for Medical Research, Copewood and Davis Streets, Camden, New Jersey 08103. Dr A. E. Greene of the institute described the collection of genetic mutant and normal control cultures. There are already about twenty different chromosome translocations in the catalogue of the repository, as well as examples of various genetic disorders. Because some people do not wish their cells to become freely available before the completion of their own experiments, Dr Greene said that lines could be held in the repository for a year before being released to purchasers.

RNA SYNTHESIS

Chopping the Chain

from our Molecular Genetics Correspondent

SEVERAL upsets in the mechanisms thought to control RNA synthesis have resulted from research on the transcription of the small DNA phage T7 and the latest is reported by Dunn and Studier in the May issue of the *Proceedings of the National Academy of Sciences* (**70**, 1559 ; 1973). The RNA polymerase of *Escherichia coli* itself transcribes T7 DNA *in vitro* from an initiation point close to the left end to a termination site about 20% of the distance along the phage. But the RNA product is much larger than the RNA molecules which are produced *in vivo* during the early stages of infection in *E. coli* cells. (Later, in infection, an RNA polymerase specified by this part of the T7 DNA transcribes the later genes of the phage located to the right.)

In agreement with previous results, Dunn and Studier found that in the absence of any factors *E. coli* RNA polymerase transcribes the entire early region of the phage into one RNA molecule. They found that addition of the rho factor, which is thought to terminate RNA synthesis within the bacterial cell, causes smaller RNA molecules to be produced. But these RNA chains appear to correspond to the very left end of the early region so that they must result from termination of transcription shortly after it has begun. Analysis by electrophoresis on gels showed that these RNAs do not correspond to the molecules produced *in vivo* in early infection.

By testing extracts of uninfected *E. coli* cells for a factor which would change the products of transcription *in vitro* so that they appear the same size as those made *in vivo*, Dunn and Studier set up an assay system to isolate the factor(s) which terminate synthesis of T7 RNA. Most extracts of *E. coli*, of course, did not change the size of the RNAs made by the polymerase. But one fraction reduced the RNA chains to the size seen during infection *in vivo*, and the "sizing factor" which possesses this activity was then purified from the crude fraction. On gels in the presence of SDS it separated into two polypeptide chains, with molecular weights of about 30,000 and 40,000.

That the sizing factor is responsible for the pattern of RNA synthesis seen within the cell is suggested by experiments using a T7 variant with a deletion ; in the presence of the sizing factor, the deletion influences *in vitro* synthesis in exactly the same manner as transcription *in vivo*. When rho factor is added to *in vitro* incubations to terminate RNA synthesis, it must be added before RNA polymerase reaches the termination site on DNA. Its action is therefore to halt synthesis at this site. But the sizing factor can act either during or after RNA synthesis. Because the same results are obtained when the RNA product of transcription is isolated and then treated with sizing factor, its action seems to be that of a nuclease.

RNA synthesis starts with a triphosphate, which remains at the 5′ end of the chain. To confirm that the small RNA products produced by sizing factor are cleaved from the larger product of transcription, Dunn and Studier examined the 5′ termini of the chains. Of the eight chains isolated after treatment with sizing factor, only three contained a γ-P^{32} label, two in ATP and one in GTP. This suggests a model in which there are three initiation sites located at the far left of T7 DNA, all used with about equal efficiency by the *E. coli* polymerase. The enzyme proceeds from any one of these sites to a point about 20% along the molecule ; sizing factor cleaves the RNA chain at specified points, either during or after its synthesis, to generate the additional RNAs found *in vivo*. Each RNA chain has five sites at which the sizing factor acts.

The most interesting implication of these results is that, because the factor was extracted from uninfected *E. coli*, another mechanism besides termination exists for controlling RNA production. With T7 RNA, the sizing factor seems to act specifically between genes. The isolation of polycistronic messengers from at least some operons of *E. coli* implies that in these instances the sequences of RNA which the factor recognizes may be absent, or at best only partially active. Although no example of messenger processing from larger precursors has been found in *E. coli*, the presence of the sizing factor activity raises the interesting possibility that this mechanism may be used. And another possible action for the factor may be to cleave ribosomal and transfer RNAs from their precursors, an activity which has so far escaped isolation. Because Dunn and Studier found that the sizing factor appears physically very similar to RNase III, an enzyme previously isolated from *E. coli* for its ability to cleave double stranded RNA sequences, its characterization—if it should prove to be RNase III—may be achieved rapidly. And because both the T7 substrates and products of its action are readily available, it may be possible to determine the sequences of the sites which it recognizes for cleavage.