CHROMATIN

Nuclear RNA Nobbled

from our Molecular Biology Correspondent

THE silence concerning the nature of Bonner's chromatin RNA has now become nothing less than deafening. It was alleged to be a defined species, some forty nucleotides in length, more than a quarter of which were dihydro-uracil. It was present in plant and animal nuclei, showed a very high degree of hybridization with the DNA, and its implication in control of transcription was boldly inferred. Heyden and Zachau (*Biochim. Biophys. Acta*, **232**, 651; 1971) have now carried out a painstaking examination of the nature of the RNA component of chromatin and the procedures used for its preparation, with dispiriting results.

Starting from calf thymus, and adhering closely to the procedure described by Shih and Bonner, Heyden and Zachau quickly encountered their first problem; the nucleoprotein pellicle—the term reserved for the scum that floats to the surface in caesium chloride centrifugation—has to be treated with pronase to liberate the nucleic acids. Pronase, being essentially a crude extract of fungus, contains a fearsome nuclease activity, which falls only during incubation, when the proteases gradually consume the nuclease components (as well as each other). Conditions were, however, found which led to the elution of two nucleic acid peaks from an ion-exchange column, as described by Shih and Bonner. One of these was DNA, and the other was identified as RNA, by the device of treating the eluted fractions with tritiated sodium borohydride. This effects the reduction of the dihydro-uracil ring to an open-chain alcohol, with introduction of tritium in sufficient quantity for counting. Before column fractionation this method failed, the RNA being evidently protected, perhaps by protein fragments.

The RNA column fractions showed in polyacrylamide gel electrophoresis only a broad smear of low-molecular weight material. If the pronase treatment of the pellicle was omitted, however, the RNA showed instead a major component migrating in the position associated with tRNA. The identification was confirmed by the demonstration in the extract of high amino-acid acceptor activity, which was substantially destroyed by the pronase treatment. Indeed, treatment of samples of tRNA with pronase under the standard conditions of the chromatin RNA preparation led to a low level of activity and a smear of degraded material exactly like that resulting from the procedure of Shih and Bonner. Gritting their teeth, Heyden and Zachau then

showed that with effort a largely nuclease-free pronase could be prepared, and its use led to the isolation of good tRNA from the chromatin; largely undergraded tRNA could also be extracted from the pellicle by treatment with urea.

They go remorselessly on to show that the endogenous tRNA hybridizes to a degree of 0.08 per cent, with its DNA, but if it is treated with crude pronase, so as to cause moderate degradation, the saturation level is increased by a factor of three, which

brings it into the range achieved with the chromatin RNA fraction, though not, it must be owned, with the enormous value of 5 per cent, reported briefly by Dahmus and Bonner (*Fed. Proc.*, **29**, 1255; 1970) for preparations from some other sources. There seems then little room for doubt that the bulk of chromatin RNA preparations is tRNA. That some small proportion is a chromatin-specific species cannot of course be excluded, but anybody seeking to demonstrate that this is so must evidently start from scratch.

Translational Control by Sex

THE well established control of gene activity in bacteria operates at the level of transcription. Repressors and RNA polymerase factors regulate different genes according to the temporary requirements of the cell. But do bacteria also regulate the level of translation of different messenger RNAs? That is, can cells choose to translate specific classes of messenger RNA molecules and to reject others? It seems that infecting bacteriophages do use such controls to appropriate the ribosomes of bacterial cells: Hsu and Weiss (*Proc. US Nat. Acad. Sci.*, **64**, 345; 1969) showed that an *in vitro* protein synthesizing system from bacteria infected by bacteriophage T4 is more receptive to T4 messenger RNA than to *Escherichia coli* messenger RNA or RNA isolated from RNA phages. This preference occurs at the level of binding of ribosomes to mRNA (Dube and Rudland, *Nature*, **226**, 821; 1970), suggesting a control of the initiation of protein synthesis. Steitz *et al.* (*Nature*, **226**, 824; 1970) also showed that the residual binding by ribosomes from T4 infected cells to RNA phage messenger displays a considerable change in the relative preference for different initiation sites on the RNA molecule. These effects of altered binding reside in a high salt wash of ribosomes; because this is known to contain the initiation factors, Steitz *et al.* suggested that altered binding may reflect a replacement of *E. coli* initiation factors by new factors specific for initiation sites of T4 mRNA.

In next Wednesday's *Nature New Biology*, Morrison and Malamy make similar arguments from quite different experiments with phage T7. These authors show that phage T7 cannot grow in male *E. coli*, that is bacteria which contain an episome (a piece of autonomous DNA) called an F factor, because most T7 messenger RNA is not translated into protein in male cells. Female cells, which lack the F factor, do support the growth of T7. Although both early and late classes of T7 messenger RNA are synthesized in male cells, only the very earliest class

of T7 proteins is found. Thus the machinery of protein synthesis in male bacteria can distinguish different classes of mRNA, an ability which would be fundamental to a translational control mechanism.

How do male cells manage this discrimination? In order to approach this question, Morrison and Malamy isolated mutants of male bacteria which do allow T7 to grow. As expected, these mutations map in the F factor. Apparently two F factor mutations (PIF⁻ 2A and PIF⁻ 2B) must be present for T7 to grow in the male cell; if only PIF⁻ 2A is present, some, but not all, late T7 proteins are made and no progeny phage are produced, although the host cell is lysed. The PIF⁻ 2B mutation has not been isolated alone. Thus the F factor seems to encode two proteins which together completely prevent the translation of late T7 mRNA.

Morrison and Malamy cannot yet say what these proteins do, although they are in favour of the interpretation that these are specific initiation factors for F factor mRNA which compete against initiation factors made by T7 specific for late T7 mRNA, but which do not compete against the normal initiation factors which serve *E. coli* mRNA and T7 early mRNA. If T7 does make specific initiation factors, it seems surprising that no conditional lethal mutants of these have been found; Morrison and Malamy argue, however, that those mutant selection procedures which have been used would not have detected them.

Although these and previous experiments suggest strongly that translation of certain classes of mRNA can be prevented, they do not yet establish a general mechanism of translational control through availability of different specific initiation factors. It is still possible, for example, that F factors and phage T4 simply produce proteins which bind to T7 and *E. coli* mRNA and thereby prevent their interaction with ribosomes. Morrison and Malamy seem to have a good system with which to provide an answer to this problem.