Colloquium on RNA Polymerase

from our Special Correspondent

Florence, November 16.

LEPETIT, the Milanese drug company, evidently believes that doctors prefer the products of drug companies which have an international reputation for sponsoring basic science. In this spirit, the company, which makes rifamycin and rifampicin, organized in Florence from November 14 to 16 a colloquium on RNA polymerase and transcription. The company made no effort to hide its immediate interest in the event, with Dr J. D. Watson as star of the show, receptions in the town hall, long addresses from civic dignitaries and even a minister up from Rome for the occasion.

The first afternoon was spent defining RNA polymerase; the complete or holoenzyme has two α chains (molecular weight 39,000), one β chain (molecular weight about 155,000), one β' chain (molecular weight about 165,000) and a molecule of sigma, σ, factor (molecular weight 90–95,000). The core enzyme lacks the molecule of σ factor. There is still doubt about the status of another polypeptide chain, the omega, ω, chain. Dr R. Burgess (Harvard) finds between 0·5 and 2 ω chains per molecule. Dr M. J. Chamberlin (Berkeley) reproducibly finds two molecules per enzyme, but Dr W Zillig (Munich) suggested that ω may be an acidic protein contaminant. Holoenzyme might therefore be $\alpha_2\beta\beta'\sigma$ or $\alpha_2\omega_2\beta\beta'\sigma$.

The second afternoon was dominated by the young men who are making the running in the sigma and terminator factor fields. Dr A. Travers (Harvard) reported that the phage T4 sigma factor is coded for by one of the immediate early *m*RNAs made during the first two minutes of infection. This sigma, he suggests, replaces the host sigma and so switches on delayed relay *m*RNA transcription. The change in sigma factors is accompanied by modification of the core enzyme resulting in less tight binding of host sigma.

Travers believes the start of delayed early transcription involves new initiation by the modified host core plus the new phage sigma, but this has still to be proved. If new initiation is required for the switch from immediate early to delayed early *m*RNA transcription two minutes after infection, the switch should be sensitive to rifampicin. Furthermore, T4 sigma should be present in the infected cells at two minutes. Travers can only detect T4 sigma five to ten minutes after infection, but that might well be because assay procedures are not yet sufficiently refined to detect it earlier. As Dr R. H. Epstein (Geneva) noted, an anti-terminator made in the first two minutes, rather than a new sigma factor, could explain the appearance of delayed early *m*RNA only after two minutes. The crucial experiment to decide the issue is to test whether delayed early *m*RNA synthesis requires new initiation; in other words, whether or not it is sensitive to rifampicin. Unfortunately, so far no one has unequivocal data about the effects of rifampicin.

Dr W. C. Summers (Yale) has isolated a T7 phage sigma factor and, as he described it, T7 has several advantages as an experimental system over T4. First, T7 has a much smaller genome; second, using temperature sensitive mutants, gene 1 has been identified as the T7 sigma factor gene. Lambda phage also makes at least one sigma factor. Dr S. Naono (Pasteur Institute) reported that during the early stages of lambda induction the *E. coli* host polymerase is modified. This modification depends on the function of gene *Q* of lambda which most people believe specifies a sigma factor.

Even though sigma factors have not yet been detected in eukaryotic cells, it is hard to resist speculating on their possible roles in cell differentiation, and Dr R. Losick (Harvard) seems on the verge of proving that sporulation in *Bacillus subtilis* involves a change in sigma factors. He knows that RNA polymerases from vegetative and sporulating cells differ in their ability to transcribe the DNA of phage φe; he now has to prove that the difference resides in different sigma factors.

The terminator factor ρ that Dr J. Roberts (Harvard) has isolated from *E. coli* complements the sigma factors. By a totally obscure mechanism, it ensures that transcription is halted at the correct site so that read through from one transcriptional group to another does not occur prematurely. In *in vitro* systems, addition of ρ, a protein of molecular weight about 200,000 which is not a nuclease, results in synthesis of RNAs of about the same chain length as those made *in vivo*. In the absence of ρ RNAs are made which are too long. There is no evidence to suggest how ρ works, but its discovery means that much of the earlier work on RNA polymerase in cell free systems needs to be reinterpreted.

On the last day of the colloquium, Schlessinger (Washington University) described ribonuclease V, a new exonuclease which could be an *m*RNase. Ribonuclease V activity depends on the presence of GTP, ribosomes, translocation factors G and T and the actual translocation of ribosomes down an *m*RNA. Schlessinger suggested that occasionally a modified ribosome attaches to an *m*RNA and degrades it sequentially from the 5′ terminus. Dr N. Zinder (Rockefeller), on the other hand, reported experiments which suggest that polynucleotide phosphorylase may be involved *in vivo* in the degradation of RNA phage RNA.

During the session on RNA phage RNAs, Dr C. Weissmann (Zürich) reported the sequence of the first 175 nucleotides of Qβ RNA. The first AUG codon appears after sixty nucleotides, but the Zürich group believe the first initiation site may be the AUG codon starting at base 102. The coat protein cistron is certainly not the first but the second, starting around 1,400 nucleotides in from the 5′ terminus.

The animal virologists, including, I think, Professor J. H. Subak-Sharpe (Glasgow), now seem to agree that, in a concentration of about 100 μg/ml., rifampicin is an anti-pox virus agent because it binds to nascent polymerase molecules, normally destined to be wrapped up in progeny virions, and as a result prevents maturation of progeny virions. The antibiotic has no effect on virus uncoating, only slightly (20–25 per cent) depresses viral DNA synthesis and possibly viral RNA synthesis, and has no effect on viral protein synthesis. Apparently the methylaminopiperazine side chain, which distinguishes rifampicin from rifamycin, may be the only part of the rifampicin molecule involved in its anti-viral activity.