THE MECHANISM OF PROTEIN BIOSYNTHESIS*

C.J. REINECKE, *Department of Physiology and Biochemical Research, Potchefstroom University, Potchefstroom, Tvl*

SUMMARY

The process of protein biosynthesis as it emerged from results presented at the 8th International Congress of Biochemistry (1970) is reviewed. Special attention is given to results concerning the initiation, elongation and termination phases of the synthesis.

During the recent 8th International Congress of Biochemistry (held in Switzerland on 3 - 9 September 1970) a number of symposia were devoted to the biosynthesis of proteins. The aim of this article is to summarize some of the contributions to our understanding of this process, as it emerged from results presented at the congress. The results in this respect dealt mainly with protein synthesis in bacteria or bacterial systems, predominantly from *E. coli*.

Protein synthesis can be divided into three main phases, each consisting of a number of definite reactions. In the initiation phase a complex is formed between the ribosomal subunits, the initiator transfer RNA (tRNA) and the messenger RNA (mRNA) at the initiation site of the latter. During the elongation phase the information of mRNA is translated into protein as the ribosome moves from the 5′ to the 3′ end of the mRNA. The third phase, called termination, occurs when the ribosome reaches the termination signal on the mRNA, whereafter the completed protein is released into the cytoplasm of the cell. Each one of these phases will now be discussed separately. A schematic presentation of the successive reactions is shown in Fig. 1.

1. Chain Initiation

The first reaction in the process of protein biosynthesis is the attachment of the ribosome to the mRNA in a position corresponding to the beginning of the cistron to be translated into protein. This position must be in the vicinity of an AUG or GUG codon, which corresponds to the initiator N-formylmethionyl-tRNA (F-met-tRNA or met-tRNA$_F$ if this tRNA is in the unformylated form). Since these codons also occur in the internal part of the genetic message, it must be assumed that the signal specifying the beginning of the cistron is more complex than just one of these codons. Furthermore, these initiation codons are probably not located near to the 5′-terminus of the mRNA. Considerable attention was therefore given at the present congress to the primary, secondary and tertiary structure of mRNAs.

Adams[1] has analysed the 5′-end of bacteriophage R17-RNA. A fragment of 74 nucleotides long has been determined. Although this sequence contains three potential initiation codons (two AUG and one GUG), it apparently does not contain the ribosomal binding site for this mRNA. This initial section of the phage RNA is part of a tightly hydrogen-bonded loop, as is shown in Fig. 2. Adams[1] suggested that this part of the phage RNA might contain the recognition site for the RNA synthetase, although it might also have another still unknown function.

Sanger *et al.*[2] have shown that in phage R17-RNA an AUG codon is present at the beginning of the cistrons coding for the three phage-specific proteins. These AUG codons probably occur at the turn of a hydrogen-bonded loop, as is shown in Fig. 3. Whether this loop might have any physiological significance for the specificity in the binding of the mRNA to the ribosome is still unknown. If so, it might be only valid for phage RNAs. Analysis of mRNAs from bacterial and other systems must be awaited before the actual role of the secondary and tertiary structure of the mRNA in the control of the initiation of protein synthesis will become clear.

Also important in the process of chain initiation is the participation of the ribosome in the recognition of the initiation signals on the mRNA. Three distinct protein factors, actually loosely bound to certain ribosomes, have so far been recognized as being involved in this process. One of these (designated factor F3 or B) is involved in the attachment of the ribosome to the mRNA (Fig. 1, Reaction 1). Revel *et al.*[3] have purified this protein and showed that it can be fractionated into cistron-specific

*Date received: 10 December 1970.

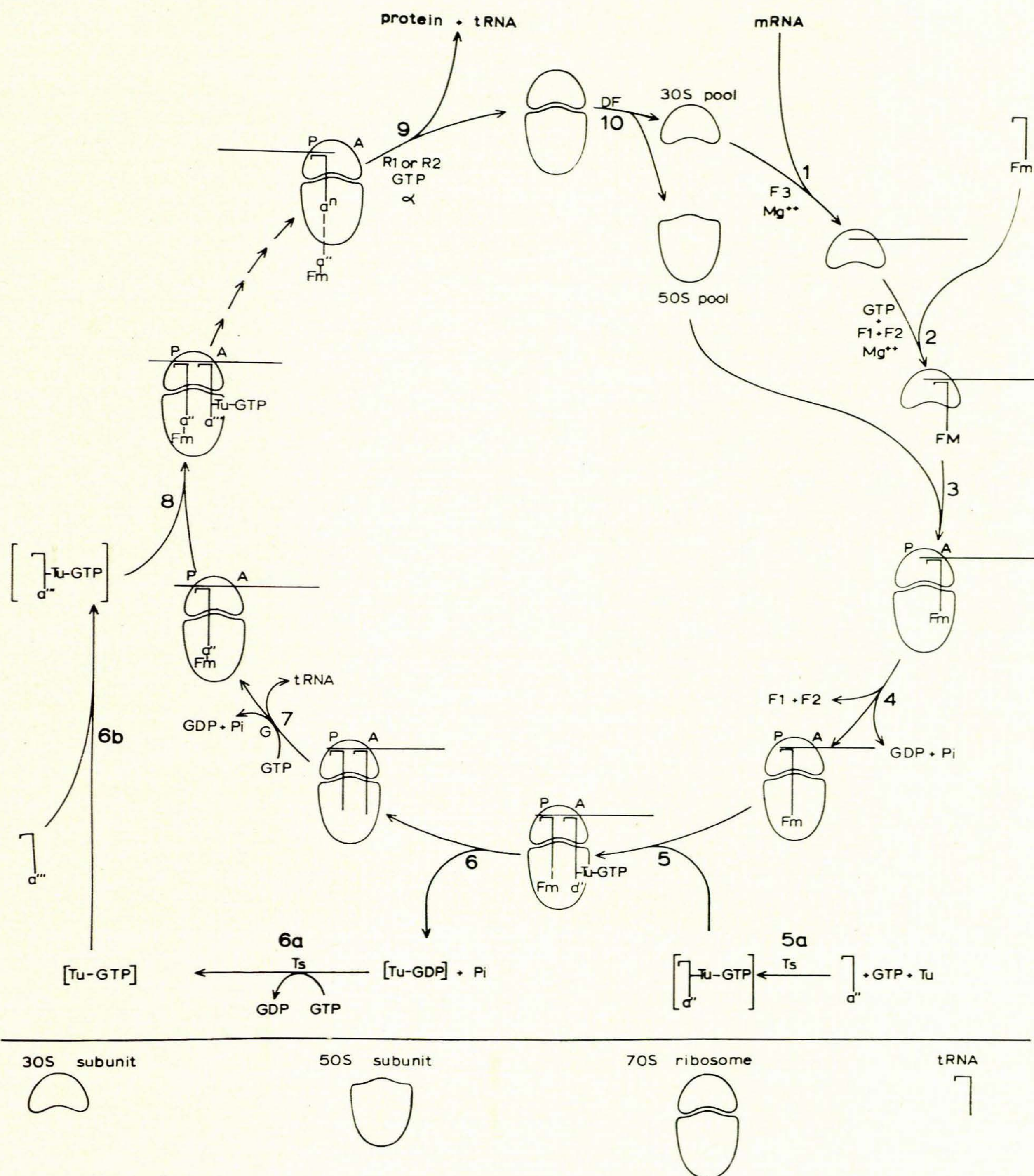

Fig. 1. Schematic presentation of the process of protein biosynthesis. Fm = N-formyl-methionine; a'' = second amino acid of the polypeptide chain; a''' = third amino acid of the polypeptide chain; a^N = the last amino acid of the polypeptide chain; A = amino acyl-site on the ribosome; P = peptidyl-site on the ribosome. The remaining abbreviations or symbols are the same as indicated in the text or in the figure.

species. This indicated that F3 can recognize selectively a proper cistron of the mRNA to be translated. The demonstration of this reaction in the process of chain initiation might provide a mechanism of gene expression control at the level of translation.

F3 could be resolved into subfractions on a DEAE-cellulose column by Gros *et al.*[4] They showed that apart from a mRNA binding function some of the fractions

```
                                              U
                                            U   U
                                            U - A
                                            U - A
                                            A - U
                                            C - G
     U—U                                    C - U
   U     C                                  G - C
    C - G                                   U   U
    C - G                                   A - U
    C - G                                   A - U
    C - G                                   U - A
    A - U                                   C - G
    G - C                                   G - C
    G - C                                   A   G
    G - U                                   G   A
    U - G                                   C - G
    G - C                                   U - A
    G .. U                                  G - C
5'-PPP - G - C                              U .. G
              A—A—C—U—U—C—C                       ............ 3'
```

Fig. 2. The untranslated nucleotide sequence at the 5′-end of R17-RNA.

```
                                 U
                               A   G
                               C - G
                               G - C
                               A - U
                               A - U
                               G - C
                               U   U
                               U - A
                               U - A
                               G - C
                               G - U
                               G - U
5' ........ A—G—A—G—C—C—U—A—A—C—C—G - U—A—C ....... 3'
```

Fig. 3. The proposed secondary structure of the ribosomal binding site in R17-RNA at the beginning of the coat protein cistron.

could also stimulate the dissociation of 70S ribosomes into 30S and 50S subunits (compare also chain termination). Similar results were presented by Ochoa,[5] and it seems therefore that F3 exhibits a dual function: binding of the mRNA to the 30S ribosomal subunit and dissociation of the ribosome into its subunits. It can, however, not be excluded that further purifications might lead to the separation of components of the present F3 factor into fractions specific for one of the above functions.

Once the 30S-mRNA complex is formed, the initiator F-met-tRNA can be bound to the complex (Fig. 1, Reaction 2). The other two initiation factors (designated F1 or A and F2 or C) are required for this reaction. This reaction, however, also requires the presence of GTP. One molecule of GTP is bound to the 30S ribosome for each molecule of F-met-tRNA present in the complex. The addition of a 50S particle to the complex causes release of GTP, presumably as GDP and inorganic phosphate (Pi). Thach and Thach[6] presented results showing that the hydrolysis of GTP occurs during the junction step, and is catalysed by F2 in the presence of both subunits (Fig. 1, Reaction 3). As soon as this reaction had taken place, F1 and F2 were released from the 70S ribosome. It was suggested that F-met-tRNA is originally bound to the aminoacyl-site (A-site) of the 30S ribosome, and is then translocated to the peptidyl-site (P-site) during the junction step (Fig. 1, Reaction 4). The energy of this translocation reaction is provided by GTP which becomes hydrolysed to GDP and Pi.

2. Chain Elongation

Once the initiator F-met-RNA is present in the P-site of the 70S-ribosome, the tRNA, carrying the second amino acid (aa), can bind to the A-site of the ribosome (Fig. 1, Reaction 5). This binding is dependent on two proteinaceous factors called Tu and Ts, present in the soluble fraction of the cell. Again GTP is required for this binding. Apparently an aa-tRNA-Tu-GTP complex is formed before binding of the aa-tRNA to the ribosome, the formation of which is catalysed by Ts (Fig. 1, Reaction 5a). As soon as the initiator and second aa-tRNA are bound adjacently to the ribosome, the first peptide bond can be formed (Fig. 1, Reaction 6). This is catalysed by peptidyl synthetase, a ribosomal protein that forms an integral part of the 50S subunit. Studies with antibiotics, presented by Vazquez *et al.*,[7] suggest that there is only one peptidyl synthetase centre on each 50S subunit.

After formation of the peptide bond, a Tu-GDP complex and Pi is released from the ribosome (Fig. 1, Reaction 6). Lengyel *et al.*[8] studied the fate of this complex. They have shown that a Tu-GTP complex is reformed in the presence of GTP, and GDP is released (Fig. 1, Reaction 6a). This is catalysed again by Ts. Subsequently an aa-tRNA-Tu-GTP complex is formed (Fig. 1, Reaction 6b) which can again be bound to the A-site of the ribosome (Fig. 1, Reaction 8). This site has become unoccupied, since the tRNA, carrying the peptide chain after formation of the peptide bond, is translocated from the A-site to the P-site in the presence of GTP and the G-factor (Fig. 1, Reaction 7). (GTP is hydrolysed to GDP and Pi during this process; the G-factor is a proteinaceous component of the soluble fraction of the cell.) The second peptide bond can now be formed, and the cycle is then repeated until the synthesis of the protein is completed.

3. Chain Termination

After formation of the last peptide bond, the complete protein is bound to the tRNA which carried the amino acid now present at the C-terminal of the protein. The termination reaction consists of two events: firstly, the recognition by the ribosome of the terminating signal on the mRNA and, secondly, the hydrolysis of the ester bond between the terminal ribose residue of the tRNA and the carboxyl group of the C-terminal amino acid.

Capecchi and Klein[9] have shown that in *E. coli* three proteins, designated R_1, R_2 and α, are involved in chain termination (Fig. 1, Reaction 9). These factors are able to recognize the terminating signals on the mRNA (the

codons UAA, UAG and UGA). R_1 mediates release of a polypeptide from the ribosome in the presence of UAA and UAG codons, whereas R_2 mediates the release in the presence of UAA or UGA codons. Two models for codon recognition were considered. In one, the terminating signal is read directly by the release factor molecule; in the other a ribosomal component interacts with the terminating signal and the resulting complex selects an appropriate release factor molecule to complete the termination reaction.

Equilibrium studies, using purified R_1, R_2 and α factors and radioactive oligonucleotides in the absence of ribosomes, demonstrated that the release factors themselves can recognize nucleotide sequences containing the chain terminating codons UAA, UAG and UGA. R_2 and α in combination show a much higher affinity for the oligonucleotides CUGA, GUAA and CUAA than for CUAG, GCAA and UCAA. Of the latter set, CUAG and GCAA showed no detectable binding and UCAA gave rise only to a low level of apparent misreading. These results were considered to indicate that a R_1-α complex has the capacity to recognize specific nucleotide sequences containing the chain terminating UAA and UGA codons.

In additon to these results of Capecchi and Klein,[9] Caskey *et al.*[10] presented evidence that α (designated S by the latter authors) facilitates the binding of R_1 and R_2 to ribosomes without specificity for only one of these factors or for trinucleotide codons. The α-factor alone has no ability to bind radioactive trinucleotides to the ribosomes, or to affect peptide release.

Hydrolysis of the nascent peptidyl-tRNA could be achieved by Caskey *et al.*[10] upon addition of R_1 and R_2 to a F-met-tRNA-AUG-ribosome complex if the reaction mixture contained 20% ethanol. This hydrolysis required potassium or ammonium ions and 70S ribosomes, while F-met-tRNA had to be in the P-site of the ribosome. The reaction was inhibited by antibiotics which also inhibit the activity of peptidyl synthetase. It was thus not possible to dissociate the release and the peptidyl synthetase activities, and it is therefore still unclear whether both R-factor and peptidyl synthetase participate in the hydrolysis of nascent peptidyl-tRNA.

After release of the completed protein from the ribosome, the latter apparently still exists in its 70S form. Algranati *et al.*[11] isolated a factor (designated DF) which can stimulate the dissociation of the 70S ribosomes into 30S and 50S subunits, which then become available for a new cycle of protein synthesis (Fig. 1, Reaction 10). Whether DF is similar to an initiation factor is still unclear (compare chain initiation). The dissociation of the 70S ribosomes mediated by DF is stimulated by GTP while ATP, CTP and UTP do not give this effect. GDP is also active but GMP is inhibitory.

DISCUSSION

From the results discussed above it is clear that the process of protein biosynthesis occurs as a cyclic process, as illustrated in Fig. 1. The ribosome enters the cycle in subunit form during chain initiation, and stays in the 70S form during chain elongation and probably chain termination, whereafter it again dissociates into subunits which then become available for a next cycle.

It is furthermore clear that a number of structural components of the ribosome, mainly of a proteinaceous nature, are involved in the translation of the mRNA. Most of these factors (e.g. F_1, F_2 and F_3) are more or less loosely bound to the ribosome, while the peptidyl synthetase forms an integral part of the structure of the 50S ribosomal subunit.

Although the main pathway of protein biosynthesis is now fairly well established, a considerable number of unsolved problems still remain. What, for example, are the functions of the great number of ribosomal proteins? Experiments on reconstituted ribosomes as developed by Nomura[12] might help to clarify this problem. Precise physical and chemical characterization of these proteins may also be expected in the near future.

Some discrepancies on the role of GTP in protein synthesis also exist:

1. Although Thach and Thach[6] found that GTP is hydrolysed during the junction step in chain initiation, other authors[4,5] noticed some hydrolysing activity in the F-met-tRNA-mRNA-30S complex.

2. An alternative function for the role of GTP in chain elongation was suggested by Leder *et al.*[13] According to these authors GTP is used for the release of the de-acylated tRNA from the P-site of the ribosome after formation of the peptide bond. As soon as the P-site becomes unoccupied the translocation reaction occurs, which would be an intrinsic function of the ribosome if the P-site is open. GTP would thus be used for release of the de-acylated tRNA instead of for the translocation of peptidyl tRNA from the A-site to the P-site.

Although most of the results described were obtained with bacterial systems, some significant results on protein synthesis in mammalian systems were also presented. Marcker and Smith[14] and Wilson[15] showed that methionyl-tRNAF also acts as chain initiator of haemoglobin synthesis in reticulocytes, although it is not formulated. The details of the latter synthesis still remain to be established, however, and much more attention to protein synthesis in eucaryotes may be expected in the near future.

I wish to thank the South African Medical Research Council, Potchefstroom University and Ciba (Pty) Ltd for financial support to attend the congress.

REFERENCES

1. Adams, J. M. (1970): Abstracts of the 8th International Congress of Biochemistry, Switzerland, p. 164.
2. Sanger, F., Jeppesen, P. G. N. and Nichols, J. L. (1970): *Ibid.*, p. 163.
3. Revel, M., Greenshpan, H., Herzberg, M., Groner, Y. and Pollack, Y. (1970): *Ibid.*, p. 193.
4. Gros, F., Lelong, J. C., Berthe'ot, F., Dondon, J. and Grunberg-Manago, M. (1970): *Ibid.*, p. 197.
5. Ochoa, S. (1970): *Ibid.*, p. 200.
6. Thach, R. E. and Thach, S. S. (1970): *Ibid.*, p. 195.
7. Vazquez, D., Stalhelin, T., Celma, M. L., Fernandez-Munoz, R., Jemenez, A., Maglot, D., Battaner, E. and Munro, R. E. (1970): *Ibid.*, p. 204.
8. Lengyel, P., Skoultichi, A., Ouo, Y., Waterson, J. and Beand, G. (1970): *Ibid.*, p. 202.
9. Capecchi, M. R. and Klein, H. A. (1970): *Ibid.*, p. 211.
10. Caskey, C. T., Scolnick, E. M., Goldstein, J., Beaudet, A., Milman, G., Rosman, M. and Tompkins, R. (1970): *Ibid.*, p. 212.
11. Algranati, I. D., Gonzales, N. S., Bade, E. G. and Baralle, F. (1970): *Ibid.*, p. 205.
12. Nomura, M. (1970): *Ibid.*, p. 187.
13. Leder, P., Roufa, D., Skogerson, L. and Bernardi, A. (1970): *Ibid.*, p. 202.
14. Marcker, K. A. and Smith, A. E. (1970): *Ibid.*, p. 193.
15. Wilson, D. B. (1970): *Ibid.*, p. 196.