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Characterization of Three Proteins Involved in Polypeptide Chain Termination

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At each stage of elongation, the growing polypeptide chain is bound to the ribosome-messenger RNA complex through the transfer RNA of the most recently incorporated amino acid residue (Gilbert, 1963; Bretscher, 1963). When the chain is complete, the last polypeptide-transfer RNA (tRNA) ester linkage is cleaved, releasing the chain from the tRNA and thus from the ribosomal complex. This hydrolysis occurs when the ribosome in the course of moving along the messenger RNA (mRNA) reaches a chain terminating signal.

The first step in elucidating the mechanism of polypeptide chain termination was to identify such signals. A purely genetic approach indicated that the codons UAA, UAG, and UGA could act as termination signals (Brenner, Stretton, and Kaplan, 1965; Weigert and Garen, 1965; Sambrook, Fan, and Brenner, 1967; Zipser, 1967). If by mutation such a codon appears, in phase, in the interior of a cistron, premature polypeptide chain termination occurs at the point of genetic alteration (Sarabhai, Stretton, Brenner, and Bolle, 1964). This codon assignment was supported by cell-free studies which showed that random U,A and U,A,G copolymers, unlike the homopolymers, directed the synthesis of polypeptides, some of which were released from the tRNAs (Bretscher, Goodman, Menninger, and Smith, 1965; Takanami and Yang, 1965; Ganoza and Nakamoto, 1966). A more direct confirmation that UAA can trigger polypeptide chain termination was the demonstration that the polyribonucleotide AUGUUUUAAA . . . directed the synthesis of the released dipeptide N-formylmethionyl (F-met-) phenylalanine (Last, Stanley, Salas, Hille, Wahba and Ochoa, 1967).

In order to further decipher the mechanism of polypeptide chain termination, the assay illustrated in Fig. 1 was devised (Capecchi, 1967a). The assay employs RNA from a mutant of the bacteriophage R17 in which the seventh codon in the viral coat protein cistron has mutated from CAG, which codes for glutamine, to UAG, which codes for release. In a cell-free amino acid incorporating system derived from *E. coli*, RNA from this mutant directs the synthesis of a small NH_2-terminal coat protein fragment, the hexapeptide F-met-Ala-Ser-Asn-Phe-Thr, which is released (Zinder, Engelhardt, and Webster, 1966). The cell-free system contains whatever factors are required for release. In order to control the release of the polypeptide chain, the synthesis had to be performed in several steps (see Fig. 1). First, the synthesis of the coat protein fragment was stopped after the phenylalanyl residue by omitting threonine from the amino acid incorporating system. The ribosome-mRNA-peptidyl tRNA complex carrying the unreleased pentapeptide F-met-Ala-Ser-Asn-Phe was then isolated from the reaction mixture by sucrose gradient centrifugation. ^{14}C-threonine was incorporated into the polypeptide chain by incubating the above complex with GTP, ^{14}C-threonyl tRNA, and purified T, one of the supernatant factors required for polypeptide chain elongation. (It was not necessary to add G-factor, the other polypeptide chain elongation factor, since this factor is carried along in the sucrose gradient, tightly bound to the ribosome-mRNA-peptidyl tRNA complex.) Now we have the routine substrate for examining the mechanism of polypeptide chain termination: the ribosome-mRNA-peptidyl tRNA complex carrying the coat protein fragment F-met-Ala-Ser-Asn-Phe-^{14}C-Thr caught just prior to release of the

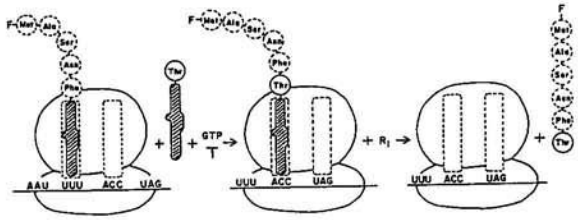

FIGURE 1. Outline of the steps required to control the synthesis of the R17 NH_2-terminal coat protein hexapeptide, F-met-Ala-Ser-Asn-Phe-^{14}C-Thr, and its release from the ribosome-mRNA-peptidyl tRNA complex. T and R_1 are *E. coli* factors required for elongation and release of the polypeptide chain respectively.

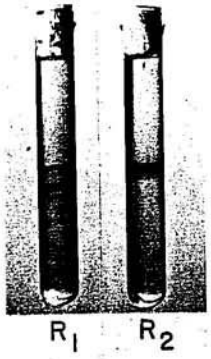

FIGURE 3. Electrophoresis of purified R_1 and R_2 (fractions VIIIa, VIIIb of Table 1) in routine 7½% polyacrylamide gels. About 9 μg of each protein was run in a Tris-glycine system, pH 8.7, at 2.6 mamp/gel for 135 min at 4°C, and stained with amido schwartz.

in parallel using the same techniques. These were isopropanol precipitation (fractions VIa, VIb), DEAE-Sephadex chromatography (fractions VIIa, VIIb), and gel filtration on columns of Sephadex G100 (fractions VIIIa, VIIIb). Table 1 shows the recovery and degree of purification of R_1 at each stage.

The homogeneity of the purest fractions of R_1 and R_2 was evaluated by polyacrylamide gel electrophoresis (see Fig. 3 and 4). For each, a single band was seen to predominate. In the case of R_1, this band accounted for approximately 80% of the protein; in the case of R_2, for approximately 99%. These estimates are supported by both routine gels (pH 8.7) (Fig. 3) and gels run in the presence of sodium dodecyl sulfate (SDS) (Fig. 4).

The data of Table 1, together with the assumptions of 80% purity of fraction VIIIa, of 44,000 for the mol wt of R_1 (see below), and of 10^{15} cells/kg of frozen *E. coli* lead to a figure of 600 R_1 molecules per cell. A calculation for R_2, which we assayed by the trinucleotide method of Caskey et al. (1968), yields the number 600 per cell also. Since we have found a ribosome content of 30,000 in cells grown under the same conditions, we estimate that there is one of each kind of release factor molecule for every 50 or so ribosomes. (Data of Leder, Skogerson, and Nau (1969) indicate that G-factor molecules, which participate in each amino acid addition to a growing polypeptide chain, have a much greater abundance, exceeding that of ribosomes. This relation is consistent with the fact that the elongation step occurs many times and the release step only once in the synthesis of a polypeptide chain.)

PHYSICAL PROPERTIES OF R_1 AND R_2

When proteins are analyzed by polyacrylamide gel electrophoresis in the presence of SDS, they run as dissociated polypeptide chains. The mobilities of the chains are inversely related to the logarithms of their molecular weights (Shapiro, Viñuela, and Maizel, 1967; Weber and Osborn, 1969). By this technique, each of the release factors was found to consist of only one species of polypeptide chain. Comparison with the mobilities obtained for a series of standard proteins indicated molecular weights of 44,000 for R_1 and 47,000 for R_2 (see Fig. 4 and 5). The behavior of R_1 on a calibrated

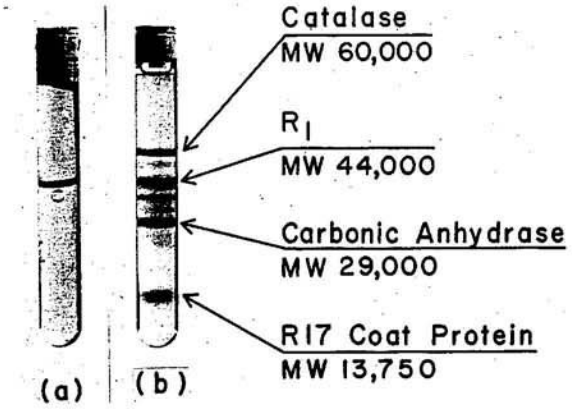

FIGURE 4. Electrophoresis of release factors in 7½% polyacrylamide gels using phosphate, pH 7.2, and 0.1% SDS. This method analyzes dissociated polypeptide chains. Gel (*a*) shows R_2 alone; gel (*b*) shows R_1 run with three of the markers used for molecular weight calibration (see text and Fig. 5). About 5 μg of R_2 and 9 μg of R_1 were used. The samples were preincubated at 37°C for 2 hr in the presence of 0.14% SDS and 1.3% 2-mercaptoethanol. They were run at 8 mamp/gel for 265 min at room temperature and stained with Coomassie Brilliant Blue.

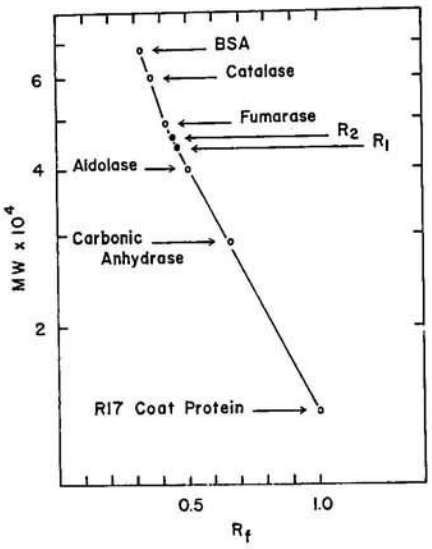

FIGURE 5. Calibration curve from the polyacrylamide gel electrophoresis of protein standards in SDS (method of Fig. 4). The molecular weights of dissociated polypeptide chains are plotted on a log scale as a function of mobility relative to that of R17 coat protein. The interpolated mobilities of R_1 and R_2 indicate molecular weights of 44,000 and 47,000, respectively.

sucrose gradient (Capecchi, 1967a) and of both factors on Sephadex G100 columns is consistent only with the assignment of the single chain molecular weights to the native proteins.

Since the release factors themselves may recognize the termination signals in mRNA, we must ask whether they are proteins or protein-nucleic acid complexes. Previous studies, which showed that R_1 activity was not affected by incubation with pancreatic or T_1 ribonuclease (Capecchi, 1967a; Caskey et al., 1968), were suggestive but could not rule out the possibility of a nucleic acid component. Therefore, we applied the more stringent criterion of phosphorus content to purified R_1. Assaying by the method of Fiske and Subba Row (1925), we found less than one atom of phosphorus per protein molecule.

Stimulating Factor

During the course of purifying R_1 and R_2 we isolated from the DEAE-Sephadex column (and further purified on a G100 Sephadex column) a protein fraction with the following properties. First, this fraction, which we call α, gives rise to only a single band on both routine (pH 8.7) and

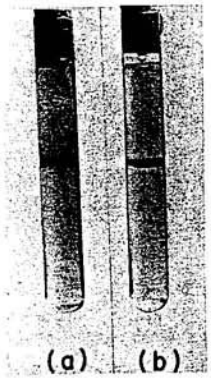

FIGURE 6. Polyacrylamide gel electrophoresis of α protein, (a) by routine method (see Fig. 3) and (b) by SDS method (see Fig. 4). About 5 μg of protein was applied for (a), about 8 μg for (b). Both gels were stained with Coomassie Brilliant Blue.

SDS polyacrylamide gel electrophoresis (see Fig. 6). Second, addition of this protein fraction to the trinucleotide chain termination assay system stimulates the rate of release of F-met mediated by either purified R_1 or R_2 (see Fig. 7). Third, in a poly U directed polyphenylalanine synthesizing system, α protein complements fractions containing G and Ts activities (see Table 2).

TABLE 2. COMPLEMENTATION PROPERTIES OF α PROTEIN IN A POLYPHENYLALANINE SYNTHESIZING SYSTEM

Protein added	Amount (μg)	μμmoles of ^{14}C-Phe incorporated*
Ts	5.0	0
G	1.0	0
α	0.4	0
Ts + G	5.0 + 1.0	0.5
Ts + α	5.0 + 0.4	0.9
G + α	1.0 + 0.4	1.8
Ts + G + α	5.0 + 1.0 + 0.4	23.5

* Background subtracted—0.5 μμmoles ^{14}C-Phe.

In addition to the components given in the above table, each 100 μl reaction mixture contained: 50 mM Tris, pH 7.8; 12 mM $MgCl_2$; 0.15 M NH_4Cl; 2 mM dithiothreitol; 2 mM phosphoenol pyruvate; 0.3 mM GTP; 2.0 μg of phosphoenol pyruvate kinase; 2.0 μg of poly U (Miles); 50 μμmoles of ^{14}C-Phe-tRNA; and 100 μg of *E. coli* B ribosomes (washed with 1 M NH_4Cl). The reaction mixtures were incubated for 10 min at 35°C and the reaction stopped by the addition of 300 μliters of 1 N NaOH. The polyphenylalanine was precipitated with 2.5 ml of 7% TCA. The final precipitate was collected on a Millipore filter and the radioactivity counted on a Packard liquid scintillation counter. Partially purified G was isolated and assayed by the method of Conway and Lipmann (1964). γ-^{32}P-GTP for that assay was a gift of J. Roberts. A crude Ts preparation was obtained from a DEAE-Sephadex column.

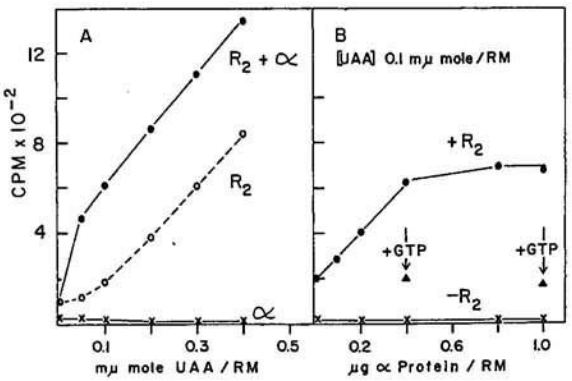

FIGURE 7. The stimulatory effect of α protein on the rate of F-met release from the ribosome-AUG-F-met-tRNA complex. Experiments *A* and *B* show UAA and α protein concentration profiles. Each 40 μliter reaction mixture (RM) contains: 25 mM Tris, pH 7.2; 30 mM $MgCl_2$; 0.10 M NH_4Cl; and 2 mM dithiothreitol. Where indicated, the reaction mixtures for Exp. *A* also contain: 0.40 μg of α protein; 0.46 μg of R_2; and the trinucleotide UAA as given. For Exp. *B*, where indicated, each reaction mixture contains: 0.10 mμmole of UAA; 0.46 μg of R_2; 4.0 mμmoles of GTP; and α protein as given. Each reaction mixture was incubated for 6 min at 23°C. The reaction was stopped by the addition of 250 μliters of 0.1 N HCl and the released F-^{3}H-met assayed using the method described by Caskey et al. (1968). The pure $tRNA_{F\text{-}met}$ used in these experiments was prepared by Oak Ridge National Laboratories under AEC-NIGMS interregional agreement and distributed by NIGMS. The spec. act. of the ^{3}H-Met was 3.1 mc/μmole.

From the behavior on polyacrylamide gel electrophoresis, this protein fraction appears to be very homogeneous, both in terms of the charge (Fig. 6*a*) and the molecular weight of the dissociated polypeptide chains (Fig. 6*b*). The molecular weight of the dissociated polypeptide chains is approximately 46,000.

A number of characteristics of the stimulatory effect of α protein on the release of F-met from the ribosome-AUG-F-met-tRNA complex are shown in Fig. 7A and 7B. Figure 7A shows the amount of F-met released as a function of added terminator trinucleotide UAA. It is clear from this figure that addition of α protein alone to the termination reaction mixture does not catalyze the release of F-met at any UAA concentration. However, addition of α protein plus R_2 results in a marked stimulation in the rate of F-met release above that obtained with R_2 alone. The degree of stimulation is particularly pronounced at low UAA concentrations where, in the absence of α protein, one can barely detect R_2-mediated release of F-met. The experiment shown was done with R_2 and UAA. The results of other experiments show that the stimulatory effect is independent of which release factor one employs and is also independent of which terminator triplet (UAA, UAG, or UGA) one uses provided UAA or UAG is used in conjunction with R_1 and UAA or UGA is used in conjunction with R_2.

In Fig. 7B the amount of F-met released as a function of added α protein in the presence and absence of R_2 is given. We see that maximal stimulatory effect is obtained by adding 0.4 μg of α protein to the reaction mixture. This small amount of protein is very nearly equivalent to the amount of pure R_2 required to saturate the system. Under R_2-saturating conditions, R_2 and ribosome-AUG-F-met-tRNA complex are present in approximately stoichiometric amounts. The ability to obtain a maximal stimulatory effect at such a low α protein concentration argues against the observed effect being due to a minor protein component of the α fraction. In Fig. 7B a curious property of the α stimulatory effect is shown. Addition of GTP (10^{-4} M) to the reaction mixture abolishes the stimulatory effect.

In conclusion, the stimulatory properties of α are very similar to those of the S protein described by Caskey, Scolnick, Tompkins, Goldstein, and Milman (this volume). In a poly U directed polyphenylalanine synthesizing system α protein exhibits the properties normally associated with Tu. In this, as in the release assay, a maximal effect is obtained at very low α protein concentration. We therefore favor the interpretation that α, S, and Tu are the same protein.

HYDROLYSIS OF THE PEPTIDYL tRNA ESTER LINKAGE

Before discussing models for the mechanism of cleavage of the ester bond between the completed but unreleased polypeptide chain and the terminal tRNA, it is important to establish at which ribosomal site the peptidyl tRNA resides. In Exp. *A* of Table 3 we show that the usual substrate for release (i.e., the ribosome-mRNA-peptidyl tRNA complex carrying the R17 coat fragment F-met-Ala-Ser-Asn-Phe-^{14}C-Thr) is sensitive to puromycin. This substrate was prepared by incubating the

TABLE 3. PUROMYCIN SENSITIVITY OF THE RIBOSOME-mRNA-PEPTIDYL tRNA COMPLEX CARRYING THE INCOMPLETE R17 COAT PROTEIN FRAGMENT F-met-Ala-Ser-Asn-Phe-^{14}C-Thr

Experiment	Incubated with	Ribosome bound hexapeptide (count/min)	Released hexapeptide (count/min)
A	—	550	2
		570	5
	R_1	50	528
		53	535
	puromycin	25	550*
		27	540*
B	—	375	7
		329	3
	R_1	312	72
		291	79
	puromycin	295	68*
		307	73*

* Released as F-met-Ala-Ser-Asn-Phe-^{14}C-Thr puromycin.

The intermediate substrate, the ribosome-mRNA-peptidyl tRNA complex carrying the R17 coat protein pentapeptide F-met-Ala-Ser-Asn-Phe, was synthesized and isolated as described by Capecchi (1967a). For Exp. *A*, a 400 μliter reaction mixture containing the above substrate was incubated for 10 min at 35°C with 20 $\mu\mu$moles of ^{14}C-Thr-tRNA (150 count/min/$\mu\mu$mole); 1 μg of T; and 40 mμmoles of GTP, in order to incorporate the ^{14}C-Thr residue into the coat protein fragment. Each reaction mixture was then incubated for an additional 10 min at 35°C in the presence of 1 μg of R_1 or 40 mμmoles of puromycin or 10 μliters of buffer. For Exp. *B*, 400 μliters of the intermediate substrate was incubated for 20 min at 35°C with 20 $\mu\mu$moles of ^{14}C-Thr-tRNA and 40 mμmoles of GMPPCP (an analog which competes with GTP). After this incubation, each reaction mixture was incubated for an additional 10 min in the presence of 1 μg of R_1 or 40 mμmoles of puromycin or 10 μliters of buffer. The released and bound coat protein hexapeptide were assayed as previously described (Capecchi, 1967b), with a variation required when hexapeptidyl puromycin was measured. This derivative, which is TCA-precipitable, can be extracted from the reaction mixture with ethyl acetate-cresol after establishing pH 5.0 with sodium acetate buffer. The unreacted hexapeptide still bound to the ribosome can then be retrieved from the aqueous phase, after base hydrolysis, by the usual assay routine beginning with TCA-precipitation.

isolated ribosome-mRNA-peptidyl tRNA complex carrying the fragment F-met-Ala-Ser-Asn-Phe with ^{14}C-Thr-tRNA, GTP, and purified transfer enzymes (see Fig. 1). If one incubates the above complex with only ^{14}C-Thr-tRNA and the GTP analog, GMPPCP (Exp. *B*, Table 3), the threonyl residue is still incorporated into the peptide chain, though at a slower rate. The resulting complex, however, is not sensitive to release by either puromycin or R_1. Experiment *A* demonstrates that, in the routine substrate for release, the peptidyl tRNA resides in the puromycin sensitive site of the ribosome. Experiment *B* demonstrates that release of the polypeptide chain cannot occur if the peptidyl tRNA is not in the puromycin sensitive site (or state) of the ribosome. The small amount of release observed in Exp. *B* is probably due to traces of GTP not removed from the complex by isolation on sucrose gradients. As a control, the substrate in Exp. *B* can be made puromycin and R_1 sensitive by adding back an excess of GTP (to overcome the effect of GMPPCP) and purified transfer enzymes.

Since the release of the polypeptide chain requires the peptidyl tRNA to be positioned on the ribosome as though it were about to undergo transfer to another aminoacyl tRNA, one can imagine two opposing models for the hydrolysis of the final peptidyl tRNA ester bond. In the first model, the release factors, R_1 and R_2, themselves possess the esterase activity. This activity would have to be ribosome dependent, for repeated efforts to demonstrate esterase activity with the purified release factors in the absence of ribosomes have failed. (The latter experiments were performed using a variety of peptidyl tRNA substrates including F-met-tRNA, polyphenylalanyl tRNA, and NH_2-terminal coat protein fragment tRNA.)

In the second model, the release factors would merely act as agents to trigger an abortive transfer of the polypeptide chain from peptidyl tRNA to water. This transfer would be catalyzed by the ribosomal peptidyl transferase. As inviting as it is to ascribe the enzymatic activity to the release factors, there does exist experimental support for the second model. Antibiotics (see Table 4) which are known to inhibit the peptidyl transferase

TABLE 4. EFFECTS OF ANTIBIOTICS ON THE R_1-MEDIATED RELEASE OF THE NH_2-TERMINAL COAT PROTEIN FRAGMENT

Antibiotic	Released hexapeptide (count/min)	Inhibition (%)
None	655	—
Chloramphenicol	295	65
Sparsomycin	177	73
Gougorotin	209	68
Tetracycline	35	95

The conditions for the preparation of the ribosome-mRNA-peptidyl tRNA complex carrying the hexapeptide F-met-Ala-Ser-Asn-Phe-^{14}C-Thr were as described in Table 3 for part *A*. The above substrate (500 μliters) was then incubated for an additional 10 min at 35°C in the presence of 1 μg of R_1 and 0.25 μmole of the indicated antibiotic. The amount of released hexapeptide was determined as previously described.

reaction (chloramphenicol, sparsomycin and gougorotin) also inhibit the release of the coat protein hexapeptide from the ribosome complex. The degree of inhibition by these antibiotics is in good agreement with the results previously reported by Scolnick et al. (1968) based on the trinucleotide release assay. The inhibition of polypeptide chain termination by these antibiotics cannot be taken as a proof of the second model, for one could argue that these antibiotics exert their inhibition by sterically hindering the access to the peptidyl tRNA bond. (A more critical experiment would be to correlate the inhibition of chain termination with the temperature sensitivity of a mutant peptidyl transferase.)

A second observation consistent with the peptidyl transferase being involved in the hydrolysis reaction is the finding that ethanol can reduce the requirements for the release of the polypeptide chain (Caskey et al., this volume). This effect of ethanol may be compared with its requirement for the formation of F-met-puromycin with the T_1 RNase fragment of F-met-tRNA as the substrate, and the 50 S ribosome subunit as the catalytic agent (Monro, 1967). Both effects may be due to facilitating the action of the peptidyl transferase by increasing the affinity of reactants for the enzymatic site on the ribosome.

READING OF THE CHAIN TERMINATING SIGNALS

Two plausible models for polypeptide chain termination can be envisioned. In the first the chain terminating signals are read; in the second they function because they cannot be read. A prediction of the second model is that polypeptide chain termination should occur in response to any unreadable codon. This prediction has been tested by depleting a cell-free amino acid incorporating system of all tRNAs capable of reading a given amino acid codon. When the ribosome reaches such a codon, polypeptide synthesis stops, but the chain is not released (Bretscher, 1968; Fox and Ganoza, 1968). Therefore, polypeptide chain termination must require the recognition of a specific sequence in the mRNA by a cellular component.

What molecules read the termination signals? An extensive search in a number of laboratories for a hypothetical tRNA-like molecule which participates in polypeptide chain termination has failed. If such a species exists, the probability of its going undetected in today's well defined in vitro chain termination systems is very small (but not zero). The absence of such molecules implies that the reading capacity belongs either to the release factors or to a ribosomal component. The difference in codon specificity exhibited by the two release factors suggests that they may have the ability to distinguish different nucleotide sequences.

We have attempted to test the hypothesis that the release factors are the agents of recognition by measuring binding constants between the purified release factors and radioactive oligonucleotides of defined sequence, using the equilibrium dialysis technique. The results to date are encouraging but not conclusive. Both R_1 and R_2 can bind oligonucleotides in the absence of ribosomes, and they have different sequence specificities. For example, the association constant between R_1 and *CUAA* under a given set of conditions (25 mM Tris, pH 7.4; 10 mM $MgCl_2$; 0.2 M KCl; 2 mM dithiothreitol; and 4°C) is 2.5 times greater than for UCAA (1.8×10^4 and 7×10^3, respectively). This is a fairly subtle distinction for the release factor to make. (These experiments were done with tetranucleotides because we could barely detect trinucleotide binding to the release factors.) Contrary to expectation, we can detect binding of R_1 to ACAA (1.4×10^4) and *CUGA* (1.6×10^4). On the other hand, conditions have been found where R_2 binds *CUGA* twice as tightly (3.3×10^4) as R_1 does. Thus a correlation between oligonucleotide binding and the known specificities of the release factors does emerge, but it is not sufficiently strict to be taken as a proof of the hypothesis.

If the binding results reflect true reading capacity, the lack of complete specificity may be due to either the difference between the environments in a ribosome and in free solution or the difference between the *natural* chain terminating signals at the ends of cistrons and the triplets UAA, UAG, and UGA. One or more of these triplets are likely to occur at least as part of any natural termination signal, but there is no reason to assume that the total signal is as short as a triplet or even that it is an integral multiple of a triplet. (Phase need not be maintained after finishing translation of a cistron.)

One must keep in mind that the capacity of the release factors to bind oligonucleotides may reflect their affinity for nucleotide sequences in the ribosome or on peptidyl tRNA in addition to or instead of an affinity for termination signals in mRNA. More work will be required before one can establish whether the release factors do indeed read the chain terminating signals.

SUMMARY

The release factors, R_1 and R_2, have been isolated in good yields and to a high degree of purity. Each was found to be composed of a single polypeptide chain with a mol wt of 44,000 for R_1 and 47,000 for R_2. R_1 contains less than one phosphorus atom per protein molecule; therefore, it does not contain any RNA.

A protein factor, α, has been isolated, which stimulates the activities of R_1 and R_2 as measured by the release of F-met from the ribosome-AUG-F-met-tRNA complex in the presence of the appropriate terminator trinucleotides. In this respect, it is similar to the S protein described by Caskey et al. (this volume). The α protein appears homogeneous on both routine and SDS polyacrylamide gel electrophoresis. Its subunit mol wt is approximately 46,000. Furthermore, in a cell-free poly U dependent polyphenylalanine synthesizing system this protein has the characteristics of Tu. The amount of α protein required to obtain a maximal effect in either the release assay or the polyphenylalanine synthesizing system is so small as to make it doubtful that either activity is due to a minor component of α protein. Therefore, we favor the interpretation that α, S, and Tu are one and the same protein. The role of α (S or Tu) in polypeptide chain termination remains to be clarified. It is interesting that the stimulating effect of α is most pronounced at low chain termination triplet concentration, where reading of the termination signal is probably rate limiting.

Evidence has been presented to show that peptidyl tRNA must be in the puromycin-sensitive site of the ribosome in order to undergo chain termination. As related to hydrolysis of the terminal peptidyl tRNA ester bond, this fact is consistent with attribution of the catalytic activity either to the release factors or to the ribosomal peptidyl transferase. The evidence to date does not exclude either model.

Finally, a hint that the release factors can read specific chain termination trinucleotide sequences has been obtained by measuring binding constants between purified release factors and radioactive oligonucleotides containing those nucleotide sequences. However, further work will be required to show whether such signal recognition is truly a function of the release factors.

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