two chromosomes, but they are replicated simultaneously. Under these growth conditions, the progeny of each chromosome appear to be segregated from the progeny of the other chromosome to maintain a situation similar to dikaryosis.

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THE RELEASE OF POLYPEPTIDE CHAINS FROM RIBOSOMES IN CELL-FREE AMINO ACID-INCORPORATING SYSTEMS BY SPECIFIC COMBINATIONS OF BASES IN SYNTHETIC POLYRIBONUCLEOTIDES*

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According to current concepts and terminology, a single continuous strand of messenger RNA (mRNA) may be transcribed from an *operon* containing coded information for two or more polypeptide chains.[1] Such messenger strands are termed *polycistronic*, and they are presumed to include signals for ending each of the polypeptide chains during translation of the genetic message by protein synthesis upon ribosomes. The termination must include the release of a carboxyl group from ester linkage to the final aminoacyl-sRNA.

It was observed that when bacteriophage f2 RNA was used as a source of mRNA in a cell-free system obtained from *E. coli*, protein was released from ribosomes to the supernatant liquid after a lag of a few minutes.[2] In contrast, when synthetic polyribonucleotides such as poly U and poly UC were used as sources of mRNA, the peptide chains remained attached to the ribosomes and were firmly attached to the ribosomal 50*S* subunit even after this had been dissociated from the 30*S* subunit by decreasing the concentration of Mg^{2+}.[3] The addition of puromycin[3] or ribonuclease (RNase) digests of aminoacyl-sRNA[4] released polypeptides from the ribosomes. This suggested that the polynucleotides used in these studies were lacking in certain nucleotide sequences which were essential for the termination and release of polypeptide chains. In view of this, we examined the behavior of syn-

thetic polyribonucleotides of various base compositions in cell-free amino acid-incorporating systems obtained from *E. coli.* It was found that a significant release of polypeptide chains into the supernatant fraction was obtained only when the polynucleotides contained U and A. Polynucleotides even of short length did not release the chains if U and A were not present, and UCI polynucleotides containing methylated bases were similarly ineffective.

Materials and Methods.—Polynucleotide phosphorylase was prepared from *Micrococcus lysodeikticus* and was used for the synthesis of polyribonucleotides from nucleoside diphosphates. Since it is difficult to synthesize large copolymers containing G, I was used instead of G in most cases where G was under consideration. The species and amounts of amino acids incorporated into polypeptide linkages were close to the values calculated from the amino acid coding assignments,[5, 16] assuming that G is equivalent to I, except in the cases of thr and ileu in systems with poly UCI (Table 8). The base compositions were determined by Dowex-1 (×2) column chromatography after alkaline hydrolysis (0.3 N KOH, 37°C for 20 hr).

$S_{20,w}$ values were determined by using a Spinco analytical ultracentrifuge with UV optics. Unless otherwise noted, polynucleotides listed in Table 1 were employed in all experiments.

Short polynucleotides: UCG copolymers, 40:40:1 and 100:100:1 by molar ratios, were synthesized and digested with T1 RNase (Sankyo Co., 10 units per mg of polymer) for 1 hr at 37°C in 0.05 M Tris-HCl buffer, pH 7.8. RNase was removed by shaking with phenol. This procedure should produce poly-UC chains with 3′-terminal G residues, and with average chain lengths close to 80 and 200, from the respective copolymers.

Elimination of U from poly-UCI: Poly-UCI was treated with 1 M NH_2OH, pH 9.0, as described by Schuster.[6] After incubation for 30 and 60 min at 37°C, the reaction was terminated by adding 2 vol ethanol. The precipitate was washed twice with ethanol, dissolved in water, and dialyzed against water. Separate aliquots of poly-UCI were treated for 2, 4, and 6 hr under similar conditions in order to determine changes in base composition. The results of base composition analyses showed that the content of U decreased linearly with the incubation time (21% by 6 hr), in contrast to the content of C and I, which remained constant. The average numbers of U residues eliminated by incubating for 30 and 60 min were calculated from the rate measurement. Since the $S_{20,w}$ value did not change markedly, it was assumed that the treatment removed some of the uridylate groups from the strands.

Polynucleotides containing N^1-methylated bases: To a solution of poly-UCI (6 mg/2 ml) were added 0.25 ml tri-n-butylamine and 0.1 ml of C^{14}-dimethylsulphate.[7] The mixture was vigorously shaken for 10 and 20 min at 0°C, following which the polymer was precipitated by adding 2 vol ethanol, washed twice with ethanol, dissolved in water, and dialyzed against water. The approximate numbers of methylated bases in the polymers were estimated from the specific radioactivity.

Assay procedures for chain release: S-30 fraction was prepared from *E. coli* B or CR63 (Su 1^- and Su 1^+, respectively) cells according to Nirenberg and Matthaei.[8] An equimolar mixture of 15 C^{14}-amino acids (excluding gluN, aspN, cySH, met, and try) was prepared and supplemented with equimolar amounts of nonradioactive gluN, aspN, cySH, met, and try. The specific activity of each amino acid was adjusted to 10^3 cpm/mμmole. The reaction mixtures contained, in a final volume of 1.0 ml, 2 μmoles ATP, 0.2 μmole each CTP and GTP, 8 μmoles phosphocreatine, 0.02 mg creatine kinase, 0.1 μmole spermine, 60 μmoles NH_4Cl, 16 μmoles Mg-acetate, 5 μmoles mercaptoethanol, 50 μmoles Tris-HCl, pH 7.8, 0.02 μmole each of C^{14}-amino acid mixture, and 0.5 ml S-30 fraction (about 4 mg ribosomes). After preparing the reaction mixture, 3 OD units (at 260 mμ) of synthetic polynucleotides were added and the mixture was incubated for 10 min at 37°C, unless otherwise noted. The reaction was terminated by chilling and adding 11 ml of cold standard buffer. The solution was transferred to the centrifuge tube of a Spinco 40 rotor. After centrifugation at 40,000 rpm for 2 hr, the supernatant upper half was removed by a capillary pipette, and aliquots were pipetted from it (*soluble fraction*). Most of the lower half was discarded, and 1 ml of solution was allowed to remain at the bottom. The ribosomal pellet at the bottom was resuspended in this (*ribosome fraction*). Trichloroacetic acid (TCA) was added to a final concentration of 10% to both fractions, and after standing for a few hours in the cold, they were brought to 90°C for 10 min, and then cooled in an ice bath. The precipitate was mounted on a Millipore filter, washed with cold 10% TCA, dried, and the radioactivity was counted.

Assay procedures for individual amino acid incorporation: The reaction mixtures were the same as those described above, except that instead of the C^{14}-amino acid mixture, 0.02 μmole of the individual C^{14} (or S^{35} in the cases of cySH and met)-amino acid and 0.02 μmole each of 19 non-radioactive amino acids were added. The specific activity was adjusted to 4×10^3 cpm/mμmole for each amino acid, and the total amount of the reaction mixture per tube was 0.5 ml. Following incubation for 30 min at 37°C, the reaction was terminated by adding TCA to a final concentration of 10%. After standing for a few hours, the hot-TCA-insoluble fraction was counted for radioactivity as described above.

Results.—The various polynucleotides listed in Table 1 were added to reaction mixtures containing the *E. coli* B cell-free system, and the distributions of C^{14}-activity in the ribosome and soluble fractions were determined after 10 min incubation. As shown in Table 2, only the four polynucleotides containing U and A

TABLE 1

BASE COMPOSITION ANALYSES AND SEDIMENTATION COEFFICIENTS OF SYNTHETIC POLYRIBONUCLEOTIDES

Polymer	Base Composition (% Molar Ratio) C	U	A	G (or I)	$S_{20,w}$
UA		79	21		14.2
UC	34	66			16.3
UG		59		41	8.6
UI		69		31	12.5
CA	71		29		17.7
CI	63			37	4.8
AI			55	45	10.4
UAC	36	48	15		9.1
UAI		54	17	29	14.4
UCI	39	32		26	4.3
CAI	60		13	26	6.0
CUAI	30	20	12	37	3.4

TABLE 2

DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY VARIOUS POLYNUCLEOTIDES BETWEEN RIBOSOMES AND SUPERNATANT IN *E. coli* B CELL-FREE SYSTEMS

Assay procedures are described in *Materials and Methods.* Liberation of polypeptide chains due to specific composition of polynucleotides is shown by underlining.

Polymer	Expt. 1 (Cpm above Control) I, Ribosomes	II, Supernatant	II/I (%)	Expt. 2 (Cpm above Control) I, Ribosomes	II, Supernatant	II/I (%)
UC	13,290	192	1.4	14,815	218	1.5
UG	6,972	524	7.4	9,468	881	9.3
UI	9,752	1,066	10.9			
UA	5,008	2,234	<u>44.5</u>	7,200	3,329	<u>46.3</u>
CI	1,854	178	9.6	2,125	176	8.4
CA	5,134	448	8.8	6,261	752	12.0
AI	956	86	9.0	1,183	93	7.9
UAI	4,328	2,782	<u>64.3</u>	4,476	2,789	<u>62.3</u>
UCI	13,990	815	5.8	13,586	435	3.2
CAI	5,598	651	11.6	5,478	493	9.0
UAC	15,273	3,508	<u>23.0</u>	14,457	3,011	<u>20.7</u>
UCAI	5,654	1,442	<u>25.6</u>	5,435	1,137	<u>20.9</u>
Control	218	95		198	90	

showed significant release of radioactivity to the supernatant; poly-UA and -UAI showed especially high values. In the contrasting cases of all the other polymers, the radioactivity found in the supernatant was around 10 per cent or less of that recovered in the ribosome fraction. It was unlikely that such differences were caused by the different solubility of the peptide chains synthesized, because when puromycin was added to the reaction mixture containing poly-UCI, a significant increase was obtained in the per cent of radioactivity which was released from ribo-

TABLE 3

EFFECT OF PUROMYCIN AND PANCREATIC RNASE ON THE DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY POLY-UCI IN *E. coli* B CELL-FREE SYSTEMS

After incubation for 10 min at 37°C with poly-UCI, indicated amounts of puromycin or pancreatic RNase were added and incubation was continued for 2 min at 37°C. Distribution of radioactivity in both ribosomes and supernatant was determined as described in *Materials and Methods*.

Incubation condition	I, Ribosomes (cpm above control)	II, Supernatant (cpm above control)	II/I (%)
No addition	14,584	845	5.7
+ 2 μmoles puromycin	9,809	4,932	50.3
+ 5 μmoles puromycin	8,760	5,449	62.2
+ 5 μg pancreatic RNase	13,381	965	7.2
+ 10 μg pancreatic RNase	13,084	1,030	7.9

somes to the supernatant (Table 3). The addition of pancreatic RNase to the system did not change the distribution of radioactivity, showing that the presence of any RNase possibly existing in the cell-free extract did not affect the results. The effect of chain length of mRNA was tested by using short polynucleotides averaging 80 and 200 residues in chain length and polynucleotides with some U eliminated from their strands. In neither case was a significant increase of radioactivity due to the modification observed in the soluble fraction. Polynucleotides containing N^1-methylated bases within the strand were also tested. It was thought that the presence of such bases within the strand might interrupt polypeptide synthesis with possible chain release because such bases cannot form hydrogen bonds with other bases. However, no increase in soluble radioactivity was produced by the modification (Table 4).

TABLE 4

DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY MODIFIED POLYNUCLEOTIDES IN *E. coli* B CELL-FREE SYSTEMS

Polymer	I, Ribosomes (cpm above control)	II, Supernatant (cpm above control)	II/I (%)
UCG(100:100:1)	21,590	414	1.9
UCG(100:100:1), treated with T1-RNase	4,856	113	2.3
UCG(40:40:1)	25,605	506	2.0
UCG(40:40:1), treated with T1-RNase	2,940	61	2.1
UCI, 12.6S*	14,985	850	5.7
UCI, one U removed per 100 bases† 11.5S*	7,400	307	4.2
UCI, one U removed per 50 bases† 6.3S*	2,546	64	2.5
UCI, one base methylated per 120 bases†	4,910	137	5.0
UCI, one base methylated per 35 bases†	377	—	—

Assay procedures are described in *Materials and Methods*.
* $S_{20,w}$ values.
† Average values.

From these experiments, we conclude that polynucleotides containing U and A must contain some code message for the termination and release of peptide chains. However, we also note that striking and consistent results were obtained only by terminating the reaction at 10 min (see also Table 6). The result was indefinite when the incubation was prolonged. This phenomenon was explored by time studies. As shown in Figure 1, poly-UA and -UAI produced high radioactivity in the supernatant at a very early stage of incubation, but following incubation, the radioactivity tended to decrease. In contrast, in such cases as poly-CA, -CAI, and -AI, radioactivity recorded in the supernatant increased gradually during incubation. We have also noted that radioactivity incorporated into polypeptides by poly-UCI and released by puromycin was diminished in the supernatant fraction by

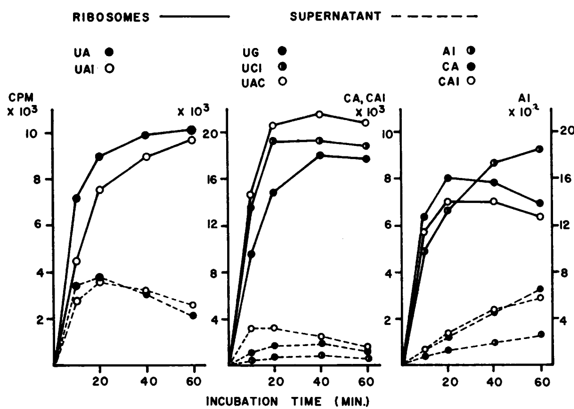

FIG. 1.—Time rates of amino acid incorporation by various polynucleotides in *E. coli* B cell-free systems.

continuing the incubation period. These results were interpreted as showing that longer incubation resulted in (1) disruption of ribosomal-sRNA-polypeptide complexes and (2) digestion of released chains by proteases presumably contained in the cell-free extract. Perhaps such proteases have a substrate specificity and attack the peptides formed and released by polynucleotides such as UA and UAI, but not those formed by CA and CAI. It was found in the case of copolymers containing U and A that increasing the proportion of A to U led to an increase of the radioactivity released in the supernatant fraction; this suggests that assuming the function to be that of a triplet, 2A1U signals chain termination and release (Table 5). It was also found that poly-UAI was more

TABLE 5

DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY POLYNUCLEOTIDES CONTAINING U AND A IN *E. coli* B CELL-FREE SYSTEMS

Polymer	Composition (Molar Ratio) A	U	I or C	I, Ribosomes (cpm above control)	II, Supernatant (cpm above control)	II/I (%)	% Frequency of: 2A, 1U	2A, 1U + 1U, 1U, 1I
AU	1	7.7		7,566	1,582	21.2	1.2	—
AU	1	5.0		7,112	2,409	33.9	2.3	—
AU	1	3.8		6,948	3,164	45.5	3.4	—
AUI	1	3.2	1.7	6,194	3,651	59.8	1.6	4.2
AUC	1	3.2	2.4	15,930	2,910	18.3	1.1	—
Control				331	101			

Assay procedures are described in *Materials and Methods.*

effective in releasing radioactivity than could be anticipated from its calculated content of 2A, 1U triplets and was more effective in this respect than poly-UAC which had almost the same content of U and A, as shown in Table 5. The UAC- and UAI-copolymers should be similar in chain-releasing effect to UA (7.7:1) if the only chain-releasing triplet is a 2A, 1U sequence, but only UAI was more effective. Since the AI and UI copolymers were ineffective (Table 2), the results therefore suggest that 1U, 1A, 1I is a second chain-releasing triplet.[9] The content of 2A, 1U, plus 1U, 1A, 1I in poly-UAI (3.2:1:1.7, Table 1) is about the same as the content of 2A, 1U in poly-UA (3.8:1) and the two copolymers had similar chain-releasing effects. The content of UAA plus UAI in the poly-CUAI preparation was 1.2 per cent, which was almost the same as the UAA content of the poly-UAC; this also agrees with the comparison of their chain-releasing potencies (Tables 2 and 6). However, chain release could be affected by other factors in addition to the frequency of the chain terminating triplets, such as the distance traveled by messenger RNA on ribosomes and the stability of messenger RNA in the reaction mixture. More experimental evidence is required to confirm the above interpretation.

Certain strains of *E. coli* (Su 1^+) are able to *suppress* an amber "nonsense" triplet which has been assigned the sequence UAG.[9, 10] An Su 1^+ strain of *E. coli*, CR63, was compared with a wild type (B), as regards the chain-terminating phenomenon in experiments similar to those in Tables 2 and 5. The results are in Tables 6 and 7; similar values were obtained for both strains. A comparison of Tables 5 and 7 suggests that the same chain-terminating triplets functioned for chain release in Su 1^+ as well as in B.

The patterns of amino acid incorporation by poly-UAI, -UCI, -UAC, and -CAI in the cell-free systems obtained from both strains were studied under similar conditions. The amounts of amino acids incorporated were compared as ratios of phe

TABLE 6

DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY VARIOUS POLYNUCLEOTIDES IN *E. coli* CR63 CELL-FREE SYSTEMS

Assay procedures are described in *Materials and Methods*. Liberation of polypeptide chains due to specific composition of polynucleotides is shown by underlining.

Polymer	I, Ribosomes (cpm above control)	II, Supernatant (cpm above control)	II/I (%)
UC	13,410	141	1.1
UG	9,752	463	4.8
UA	7,780	3,160	40.7
CI	2,376	152	6.4
CA	5,711	449	7.9
AI	1,389	124	8.9
UAI	8,782	4,689	53.5
UCI	15,664	783	5.0
CAI	8,830	599	6.8
UAC	18,718	2,920	15.6
Control	235	112	

TABLE 7

DISTRIBUTION OF C^{14}-AMINO ACIDS INCORPORATED BY POLYNUCLEOTIDES CONTAINING U AND A IN *E. coli* CR63 CELL-FREE SYSTEMS

Polymer	Composition (Molar Ratio) A	U	I or C	I, Ribosomes (cpm above control)	II, Supernatant (cpm above control)	II/I (%)	% Frequency of: 2A, IU	2A, IU + 1A, IU, II
AU	1	7.7		7,222	1,220	16.9	1.2	—
AU	1	5.0		7,866	2,329	29.6	2.3	—
AU	1	3.8		7,524	3,161	42.0	3.4	—
AUI	1	3.2	1.7	6,902	3,055	44.3	1.6	4.2
AUI	1	4.0	2.8	8,414	3,270	38.8	0.8	3.1
AUC	1	3.2	2.4	17,416	2,820	16.2	1.1	
Control				325	74			

Assay procedures are described in *Materials and Methods*.

or pro incorporation and both strains showed similar incorporation profiles, except in the case of ser with poly-UAI, which was about 50–60 per cent higher in CR63 than in B (Table 8). This was confirmed by testing other UAI copolymers with varying compositions and also by measuring time rates of incorporation. The incorporation patterns of amino acids were close to the values calculated from amino acid code assignments assuming I = G.[5, 16] The only exceptions were the incorporation of thr and ileu by poly-UCI, since all thr and ileu codes contain A (Table 8). It has been suggested that I in the coding sequences of certain sRNA molecules may exhibit ambiguity of base pairing,[13] so that a coding triplet containing I in sRNA may complement with more than one mRNA triplet, and Nirenberg and co-workers[14]

TABLE 8

INCORPORATION OF AMINO ACIDS BY POLY-UAI, UCI, UAC, AND CAI IN *E. coli* B AND CR63 CELL-FREE SYSTEMS

Assay procedures are described in *Materials and Methods*. Data for glu and asp are regarded as inaccurate, because glu ⇌ gluN and asp ⇌ aspN changes may occur during incubation. The symbol ± indicates less than 2× the amount of incorporation without copolymer; +, more than 2×. In *E. coli* B, incorporation of phe was 1.9 mμmoles by UAI, 1.1 mμmoles by UCI, 2.2 mμmoles by UAC, and that of pro was 1.7 mμmoles by CAI. The calculated values are from the following assignments: phe, UUb; leu, UUe, CUd; ser, UCd, AGb; ileu, AUb, AUA; val, GUd; ala, GCd; gly, GGd; thr, ACd, his, CAb; lys, AAe; arg, CGd, AGe; asp, GAb; glu, GAe; pro, CCd; tyr, UAb; met, AUG; try, UGG; cySH, UGb (*b* = U, C; *d* = A, G, U, C; *e* = A, G).[16]

	UAI (% of phe)			UCI (% of phe)			UAC (% of phe)			CAI (% of pro)		
		Found			Found			Found			Found	
	Calcd.	B	CR63	Calcd.	B	CR63	Calcd.	B	CR63	Calcd.	B	CR63
phe	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	0	0	0
leu	79	87	86	210	219	195	106	117	108	0	0	0
ser	15	19	30	176	208	194	89	93	92	6	16	17
ileu	41	48	45	0	22	26	37	47	50	0	±	±
val	84	97	92	108	128	118	0	0	0	0	0	0
ala	0	0	0	140	133	120	0	0	0	43	36	36
gly	40	31	34	86	92	87	0	0	0	19	12	13
thr	0	0	0	0	38	35	28	33	31	22	29	27
his	0	0	0	0	0	0	24	19	19	13	13	15
lys	8	14	13	0	0	0	2	5.0	5.0	2	2.5	3.1
arg	12	15	14	140	202	186	0	0	0	47	55	57
asp	15	7.5	7.5	0	0	0	0	±	±	6	+	+
glu	12	9.3	9.1	0	0	0	0	+	+	4	5.1	6.9
pro	0	0	0	228	185	176	66	59	69	(100)	(100)	(100)
tyr	31	25	26	0	0	0	36	34	36	0	0	0
met	15	24	25	0	±	±	0	±	±	0	0	0
try	22	18	17	28	26	26	0	0	0	0	0	0
cySH	47	44	49	80	73	55	0	0	0	0	0	0

have observed that yeast alanyl sRNA, containing the antiparallel sequence CGI,[15] will bind with ribosomes charged with GCU, GCC, or GCA. The present results indicate that this effect may have its counterpart in a similar ambiguity of I in synthetic polyribonucleotides used as mRNA in that I can simulate A in the messenger codes for thr and ileu. However, other amino acids containing A plus C or U in their codes, such as tyr, his, and met, were not incorporated by UCI.

Discussion.—Brenner and co-workers[9] have shown by means of genetic and biochemical analyses that "amber" and "ochre" mutants of bacteriophage T4 contain two kinds of suppressible "nonsense" (not translated as amino acids) triplets to which they assign the sequences UAG and UAA, respectively. They have suggested that these function to terminate polypeptides in protein synthesis. Nirenberg and co-workers[5] reported that the trinucleotides UAA, UAG, and UGA had no activity in binding aminoacyl-sRNA's to ribosomes. Our experiments do not supply information on base sequences, but our findings agree with the proposal by Brenner *et al.*[9] in that only polynucleotides containing UA produced release of polypeptide chains from ribosomes, and that 2A, 1U and 1A, 1U, 1I appeared to be the most probable chain-terminating triplets.

It has been reported that in bacteriophage T4D head protein,[9] in *E. coli* alkaline phosphatase,[10] and in bacteriophage f2 coat protein,[11] a gluN or try residue in the wild-type protein is replaced by ser in the mutant protein produced in suppressor cells. It was also shown that a suppressor mutant of *E. coli* contains a special seryl-sRNA which reads the chain-terminating ("nonsense") triplet of RNA prepared from an amber mutant of the RNA bacteriophage R17 in cell-free systems as ser.[12] We closely compared cell-free systems from *E. coli* strains B (wild) and CR63 (Su 1^+) with respect to chain release and amino acid incorporation by synthetic polynucleotides. Ser incorporation was increased by about 50 per cent by poly UAI in CR63 as compared with B. This latter finding agrees with the finding by Brenner *et al.*[9] that the efficiency of synthesis of T4D head protein in intact cells of CR63 was about 60 per cent, suggesting that UAG is partially read as ser. However, our technique did not detect any difference in chain release between the B and CR63 systems. Our quantitative findings of try incorporation with UAI and UCI (Table 8) were compatible with UGG's being the only code for try.

The question of the mechanism of recognition of chain-terminating triplets in mRNA is still open. Brenner *et al.*[9] have proposed that an RNA molecule, similar to sRNA, recognizes the chain-terminating triplets and releases the polypeptide chain. This idea is supported by the fact that the chain is attached to ribosomes solely by the sRNA-binding site of ribosomes.

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*HYBRID COMPOUNDS IN NATURAL INTERSPECIFIC HYBRIDS**

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Since the more recent applications of chemical methods to the study of natural interspecific hybridization, there has been considerable interest in the theoretical question of whether or not new structural configurations occur in such hybrids; i.e., hybrid-specific products formed by the combined enzymatic complements of the parents. Many years ago, Reichert[1] called attention to the fact that hybrids occasionally exhibited unexpected flower colors. The appearance of new compounds has been suggested from chemical analyses of interspecific hybrids. Alston and Turner[2] described four hybrid-specific flavonoids in the leaves of the hybrid *Baptisia leucantha* × *B. sphaerocarpa*, but the compounds were later shown to occur in the flower petals of the latter species.[3] Other sporadic suggestions of the occurrence of such hybrid-specific substances have not been followed by definitive experimental proof of their existence. Diverse genetic studies of flavonoid compounds (the only such extensively investigated plant secondary compounds) have invariably shown that simple mendelian mechanisms govern qualitative differences in these compounds.[4] From current knowledge of the types of flavonoid compounds which are widely distributed in plants, it is possible to predict a large number of ways in which hybrid substances might occur. This question is important to systematic studies utilizing variability in the patterns of secondary compounds as systematic criteria.

We are concerned here with a number of hypothetical compounds which could be expected to exist in the following natural hybrids of *Baptisia: B. leucantha* × *B. sphaerocarpa; B. alba* × *B. tinctoria;* and *B. alba* × *B. perfoliata.* Other hybrid-type molecules may be predicted to occur in other known *Baptisia* hybrid combina-