# EVIDENCE ON A CODON RESTRICTION HYPOTHESIS OF CELLULAR DIFFERENTIATION: MULTIPLICITY OF MAMMALIAN LEUCYL-SRNA-SPECIFIC SYNTHETASES AND TISSUE-SPECIFIC DEFICIENCY IN AN ALANYL-SRNA SYNTHETASE

BY BERNARD L. STREHLER, DANIEL D. HENDLEY, AND GERALD P. HIRSCH

GERONTOLOGY BRANCH, NICHD, NIH, BALTIMORE CITY HOSPITALS, AND AGING RESEARCH LABORATORY, VETERANS ADMINISTRATION HOSPITAL, BALTIMORE, MARYLAND

Certain suggested models of cell differentiation during ontogeny have important implications for cell and organismic senescence. Thus, as pointed out by Weismann,[1] and later by Minot,[2] Pearl,[3] and Medawar,[4] aging may be, in large measure, a long-term consequence of limitations in function that occur as by-products of the processes of cell specialization that take place during normal development.

It is now apparent that differentiation and development involve selective syntheses in different cells at different times of proteins coded in different portions of the genome common to all cells of a developing organism. The general model mechanisms which have been suggested to account for such selective protein syntheses include (a) those which incorporate the concept of selective messenger synthesis and (b) those which postulate selective translation of messages as the limiting agents in differential synthesis. Models of the former category have been suggested by Bonner[5] and by Allfrey,[6] whereas a model in the second category has been suggested recently by one of us.[7] This latter hypothesis, which bears some resemblances to earlier suggestions of Stent,[8] Itano,[9] and Ames and Hartman,[10] consists of the following elements:

(1) The multiplicity of codon triplets in the genetic code signifying a given amino acid provides the possibility that a given sequence of amino acids may be specified by many alternative nucleotide sequences.

(2) The messages unique to and expressed in a cell type, a, are coded in a limited group of triplet codons, A (cell-specific set or language), whereas cell-type-b, c,..., x specific messages are coded in triplet language sets B, C,..., X, respectively.

(3) Each unique cell type is unable to translate messages unique to any other cell type because of at least one untranslatable word in all alien word sets.

(4) In cells unable to translate a given word, the limiting component is the aminoacylated sRNA corresponding to the untranslatable triplet.

Components whose deficiency in a cell would result in the lack of an appropriate aminoacyl-sRNA are: (a) the amino acid; (b) the specific sRNA; and (c) a codon-specific aminoacyl-sRNA synthetase.

This communication presents evidence we have obtained bearing on the last-named alternative. It has been shown that:

(a) Rabbit heart muscle contains several chromatographically separable leucyl-sRNA synthetases.

(b) Two of these enzymes preferentially attach leucine to chromatographically different species of sRNA.

(c) Enzyme preparations obtained from reticulocytes and from liver differ quantitatively in the pattern of leucyl-sRNA species they can activate.

(d)  There exist two or more chromatographically separable species of sRNA (rabbit) to which alanine may be attached.  One of these sRNA's is coupled to alanine by synthetases present in kidney, reticulocytes, or liver.  By contrast, alanine is readily incorporated into a second sRNA fraction only by synthetases present in liver and reticulocytes, but either not at all or very slightly by synthetases obtained from kidney.

*Methods.—Enzyme preparations:*  Except for the reticulocyte preparations, and unless otherwise indicated, all aminoacyl-sRNA synthetase preparations were made from rabbit tissues homogenized in 2.3 volumes of medium A[11] and centrifuged at 105,000 × *g* for 15 min.  To remove endogenous amino acids and part of the sRNA, 5 ml of the supernatant solutions were passed through 1.1- × 55-cm columns of Sephadex G-100 (Pharmacia, Uppsala, Sweden), equilibrated with 0.01 *M* Tris, pH 7.5 (25°), 0.01 *M* mercaptoethanol.  A 6- to 8-ml fraction (up to the beginning of the hemoglobin elution) was collected and either used immediately or frozen in liquid $N_2$.

The reticulocyte preparations were made from washed red cells taken from rabbits made anemic by injections of phenylhydrazine.[12]  The cells were stored frozen at −40°C and lysed when needed by homogenization in two volumes of 0.002 *M* $MgCl_2$.  The lysates were centrifuged and passed through Sephadex G-100 as indicated above.  The collected fraction included about 4 per cent of the hemoglobin.

*sRNA preparation:*  Transfer RNA was isolated from rabbit heart, liver, or reticulocytes by blending 100 gm of tissue with 300 ml of 0.01 *M* Tris, pH 7.5, 0.005 *M* $MgCl_2$, 0.0005 *M* ethylenediaminetetraacetate (EDTA).  The homogenate was immediately shaken with 300 ml of redistilled phenol for 1 hr at room temperature.  After centrifugation, the RNA was precipitated from the aqueous phase with 1/10 volume of 20% potassium acetate and 2 volumes of 99% ethanol.  The sRNA was extracted from the precipitate by washing three times with 25-ml portions of 1 *M* NaCl, 0.05 *M* in Tris, pH 7.5.  After precipitation with ethanol-potassium acetate, the sRNA was chromatographed on *O*-(diethylaminoethyl) cellulose (DEAE-cellulose), eluting with a linear NaCl gradient 0.01 *M* in Tris, pH 7.5.  Fractions in the second-eluted RNA peak were pooled and freed from bound amino acids by incubation in 0.1 *M* sodium carbonate-bicarbonate buffer, pH 10, at 37°C for 30 min.[13]  A mixture of such sRNA preparations, containing 43% liver, 34% heart, and 23% reticulocyte sRNA, was used for incorporation experiments with leucine.

*Aminoacylation of sRNA and methylated albumin kieselguhr (MAK) column chromatography:*  In addition to activating enzymes, the reaction mixture contained 1 mg transfer RNA mixture, 0.005 *M* adenosine 5′-triphosphate (ATP), 0.003 *M* glutathione (GSH), 0.01 *M* $MgCl_2$, 0.1 *M* $K^+$ cacodylate buffer, pH 7.0, 0.001 *M* EDTA, and 0.0125 m*M* L-leucine uniformly labeled with $C^{14}$ or 4,5-$H^3$.  Total volume, 1.0 ml.  After incubation at 28°C the reaction was stopped by mixing with 1.0 ml water-saturated phenol, and chilling to 0°C.  Reaction mixtures to be cochromatographed were then mixed together and shaken 10 min.  The RNA was precipitated from the aqueous phase two times with 2.5 volumes of ethanol and chromatographed at 4°C on a 6-gm celite column containing methylated serum albumin, according to the procedure of Yamane and Sueoka,[14] using 300 ml of a linear NaCl gradient (0.3–0.6 *M*).  Aliquots (2.0 ml) of the eluate were mixed with 18 ml of a water-miscible scintillator mixture,[15] and counted in a scintillation counter.  The results plotted have been corrected for background and "spill" of $C^{14}$ counts into the $H^3$ channel.

*Assay of aminoacyl-sRNA synthetase activity:*  Aliquots (0.05 or 0.025 ml) of reaction mixtures were spotted onto filter paper disks, which were then placed on drops of 10% trichloroacetic acid (TCA) containing carrier amino acids.  After 20 min at 0°C the disks were washed individually and consecutively with 5-ml volumes of 10% TCA, then with 1:1 ethanol:ether, dried, and counted in a toluene scintillator solution.

*Experimental Results.—Evidence that a tissue contains several chromatographically separable aminoacyl-sRNA synthetases for the same amino acid:*  Figure 1 shows the relative rates of leucine incorporation into a mixture of sRNA's by fractions of a crude heart muscle enzyme preparation resolved by chromatography on a DEAE cellulose column.  Three separate peaks of activity are apparent.
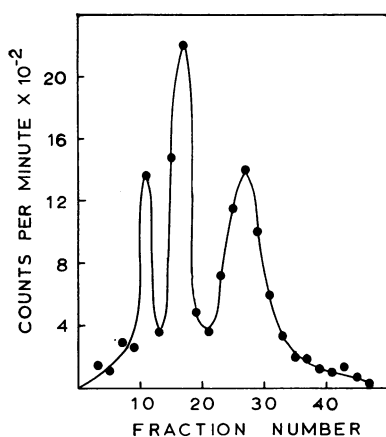
FIG. 1.—Distribution of leucyl-sRNA synthetase activity in fractions of heart supernatant eluted from DEAE cellulose. Three ml of heart $105,000 \times g$ supernatant (see *Methods*) were passed through a Sephadex G-25 column, applied to a $1.0 \times 13.5$-cm DEAE-Cl$^-$ column, and eluted with 225 ml of a non-linear NaCl gradient $0$–$0.45$ $M$ NaCl, $0.05$ $M$ in Tris-Cl$^-$, pH 7.5, $0.01$ $M$ mercaptoethanol. Assay: 50-$\mu$l aliquots of enzyme fractions were incubated with 20 $\mu$g rabbit liver sRNA and (final concentrations of) $0.01$ mM L-leucine-H$^3$, 5 c/mmole, $0.005$ $M$ ATP, $0.005$ $M$ MgCl$_2$, $0.05$ $M$ potassium cacodylate buffer, pH 7.0, $0.003$ $M$ GSH, $0.0005$ $M$ Na$_2$EDTA. Total volume $= 75$ $\mu$l. After 30 min at 28°C, a 50-$\mu$l aliquot was applied to a filter paper disk, precipitated, washed, and counted as described in *Methods*.

*Evidence that chromatographically different synthetases charge different leucyl-sRNA species:* The enzyme fractions contained in the peak of activity eluting first from the column were combined and designated enzyme 1, while fractions eluting late were combined as enzyme 2. Their respective capacities to incorporate labeled leucine into different sRNA fractions was determined by cochromatography on a MAK column, as described in the *Methods* section. The results of one such experiment are shown in Figure 2. This graph shows that enzyme 1 labels predominantly sRNA species that emerge late from the chromatographic column (peak III RNA material) and conversely that enzyme 2 labels predominantly sRNA species which elute early.

Further evidence that a single amino acid species is attached to different kinds of sRNA by different enzymes was afforded by the results depicted in Figure 3. This experiment shows that crude enzyme preparations obtained from reticulocytes are relatively low, when compared to those from liver, in their ability to incorporate label into the peak III component.
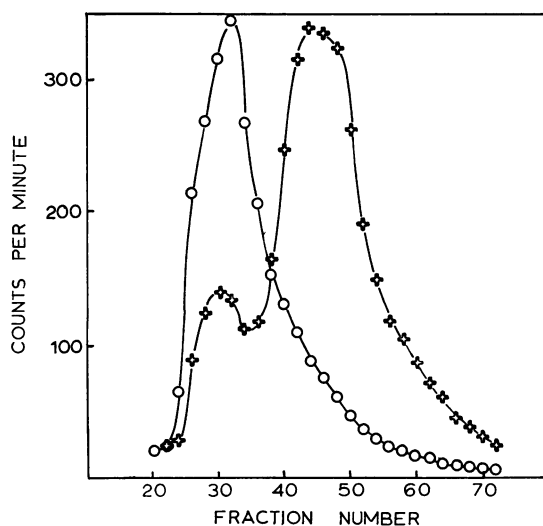


FIG. 2.—Elution patterns of leucyl-sRNA's charged by two DEAE fractions from heart, then mixed, and cochromatographed on a MAK column. $+\ +$, H$^3$-leucyl-sRNA, formed by enzyme 1. O-O, C$^{14}$-leucyl-sRNA, formed by enzyme 2. Enzyme 1 represents pooled fractions 10–12 and enzyme 2 represents fractions 24–31 (Fig. 1). The pooled fractions were concentrated by precipitation with 80% saturated (NH$_4$)$_2$SO$_4$, pH 7.2, $0.01$ $M$ in mercaptoethanol. Charging of the sRNA by the enzyme fractions is described under *Methods*.
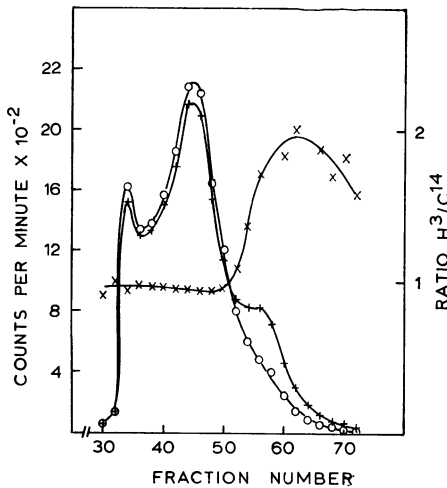
FIG. 3.—Elution patterns of leucyl-sRNA's charged by crude liver and reticulocyte enzyme preparations. O-O, C$^{14}$-leucyl-sRNA formed by reticulocyte enzyme; +-+, H$^3$-leucyl-sRNA formed by liver enzyme. X-X, ratio, H$^3$/C$^{14}$. The enzymes were derived from homogenate supernatants prepared as described under *Methods* up to the Sephadex G-100 step. Both preparations were desalted on a Sephadex G-25 column, treated with protamine to remove endogenous sRNA, and precipitated with (NH$_4$)$_2$SO$_4$, according to the procedures of Allen and Schweet.[26]
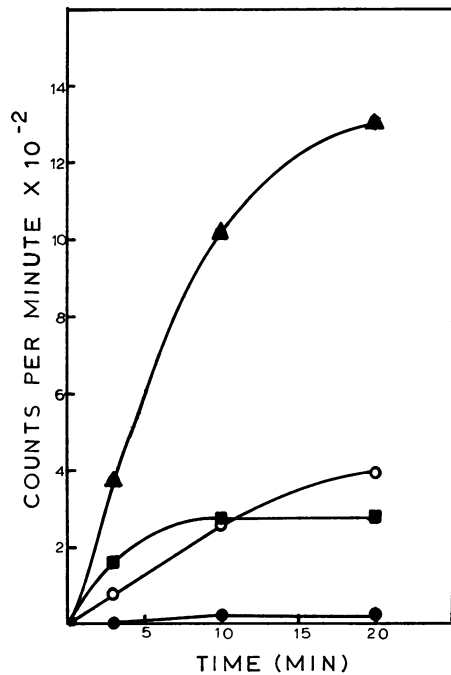
FIG. 4.—Incorporation of H$^3$-alanine into sRNA fractions 125 and 150 by crude enzyme preparations from liver and kidney. The data were taken from Table 1. O-O, fraction 125 sRNA, kidney enzyme; ▲-▲, Fraction 125 sRNA, liver enzyme; ●-●, fraction 150 sRNA, kidney enzyme; ■-■, fraction 150 sRNA, liver enzyme.

*Evidence that tissues differ qualitatively in their codon-specific aminoacyl-sRNA synthetase complements:* The results shown in Figure 3 represent the first evidence suggesting that mammalian cell differentiation entails differences in the pattern of activation of different sRNA species for the same amino acid. That crude enzyme preparations from different cell types may differ qualitatively in their ability to charge chromatographically distinct sRNA fractions for the same amino acid is illustrated by evidence obtained as follows.

Deacylated sRNA species from rabbit heart muscle, liver, kidney, and brain were mixed, chromatographed according to the method of Kelmers et al.,[16] and the distribution of acceptor activity for each of 16 amino acids was determined using a crude rabbit liver enzyme preparation. Two or more separable discrete peaks for ten different amino acids were then tested against enzyme preparations obtained from five rabbit tissues (brain, reticulocytes, heart muscle, kidney, and liver) as described in the *Methods* section. Indications of tissue differences in the ratio of label incorporation into appropriate pairs of sRNA fractions were observed for leucine, proline, alanine, arginine, and histidine. Of these, alanine incorporation into separate sRNA fractions by enzymes obtained from different tissues appeared to be qualitatively different.

Table 1 shows the rate of incorporation of alanine label into fractions 125 and 150 sRNA by reticulocyte, liver, and kidney enzymes. Figure 4 illustrates the differences between the results using kidney and liver enzymes, respectively. For the sake of completeness we have included data for all three incubation periods measured. It is clear, however, that the rate of incorporation drops off significantly between 3 and 20 minutes and that the three-minute values are the most valid estimates of relative enzyme activities. The column at the extreme right in Table 1 represents the radioactivity incorporated into fraction 150 (after subtraction of blank values) expressed as per cent of the incorporation into fraction 125. By comparing these values for the three-minute incubations, it may be seen that the per cent labeling of fraction 150 sRNA by the kidney enzymes is only 0 to 14 per cent of that of the liver or reticulocyte enzymes.

Control experiments as detailed in Table 2 rule out selective lysis of sRNA or amino acid contaminants or interconversions as artifactual sources of the observed tissue differences. The data are derived from incubations run on the same day and under the same conditions as those in Table 1 (using the lower alanine concentration), and are to be compared with them. Line 2 demonstrates that a 20-minute preincubation of fraction 150 with kidney enzyme did not prevent the incorporation of counts into that fraction by reticulocyte enzyme. The preincubation with kidney enzyme reduced the 150/125 sRNA ratio obtained with reticulocyte enzyme by 50 per cent; but this is far from sufficient to account for the observed tissue differences in the three-minute incubations of Table 1.

## TABLE 1
### INCORPORATION OF ALANINE INTO sRNA FRACTIONS BY RABBIT TISSUES

| Enzyme | Incubation time (min) | sRNA 125 (cpm) | sRNA 150 (cpm) | Blank* (cpm) | 125-Blank (cpm) | 150-Blank (cpm) | Ratio 150/125 × 100 |
|---|---|---|---|---|---|---|---|
| Fresh kidney | 3 | 140 (14) | 62 (2) | 58 (1) | 82 | 4 | 5 |
| | 10 | 310 (24) | 82 (4) | 54 (0) | 256 | 28 | 11 |
| | 20 | 455 (53) | 85 (6) | 60 (11) | 395 | 25 | 6 |
| Fresh liver | 3 | 437 (10) | 220 (1) | 62 (1) | 375 | 158 | 42 |
| | 10 | 1074 (70) | 327 (43) | 51 (10) | 1023 | 276 | 28 |
| | 20 | 1352 (4) | 324 (42) | 47 (6) | 1305 | 277 | 21 |
| Frozen kidney† | 3 | 196 (37) | 56 (18) | 54 (1) | 142 | 2 | 1 |
| | 10 | 453 (102) | 74 (17) | 63 (6) | 390 | 11 | 3 |
| | 20 | 556 (210) | 80 (20) | 54 (5) | 502 | 26 | 5 |
| Frozen liver† | 3 | 331 (20) | 154 (1) | 53 (1) | 278 | 101 | 36 |
| | 10 | 867 (79) | 233 (46) | 49 (1) | 818 | 184 | 23 |
| | 20 | 1116 (305) | 223 (65) | 59 (2) | 1057 | 164 | 16 |
| Frozen reticulocytes† | 3 | 447 (15) | 216 (33) | 54 (2) | 393 | 162 | 41 |
| | 10 | 1143 (62) | 265 (76) | 46 (6) | 1097 | 219 | 20 |
| | 20 | 1387 (268) | 276 (96) | 48 (2) | 1339 | 228 | 17 |
| Frozen kidney—higher alanine concentration | 3 | 1096 | 150 | 155 | 941 | 0 | 0 |
| | 10 | 2511 | 284 | 137 | 2374 | 147 | 6 |
| | 20 | 3113 | 283 | 157 | 2956 | 126 | 4 |
| Frozen liver—higher alanine concentration | 3 | 1687 | 430 | 80 | 1607 | 350 | 22 |
| | 10 | 4459 | 985 | 77 | 4382 | 908 | 21 |
| | 20 | 6835 | 986 | 135 | 6700 | 851 | 13 |

Incorporation of $H^3$-alanine into sRNA fractions 125 and 150 from a liquid partition column prepared according to Kelmers et al.[16] was measured using crude synthetase preparations prepared as described in Methods. One hundred mg of a mixture of equal proportions of sRNA from rabbit heart muscle, brain, liver, and kidney was deacylated, applied to a column 1.2 cm × 8 feet, and eluted with 3 liters of a linear gradient of NaCl (0.3–0.7 M) and $MgCl_2$ (0–0.01 M), 0.05 M in Tris pH 7.4. Ten-ml fractions were collected. Aliquots (0.05 ml) of fractions 125 or 150 were incubated with 0.01 ml of the indicated enzyme, 1 × 10⁻⁷ M $H^3$-DL-alanine, 40 c/mmole, and ATP, buffer, $MgCl_2$, EDTA, and GSH, as in Fig. 1. The final volume was 0.1 ml. The higher alanine level was 1.5 × 10⁻⁶ M. After the indicated incubation time at 28°C, 0.025-ml aliquots were precipitated on filter paper disks and counted as described in Methods. Each value represents an average of corresponding time points from duplicate assay tubes. The figures in parentheses indicate one half the range.

* The "blank" column indicates counts incorporated into tubes containing 0.5 M saline in place of the sRNA fraction.

† "Frozen" enzyme refers to preparations from a different animal which were frozen in liquid $N_2$ until used.

TABLE 2

| Enzyme | Incuba-tion time (min) | sRNA 125 (cpm) | sRNA 150 (cpm) | Blank (cpm) | 125-Blank (cpm) | 150-Blank (cpm) | Ratio 150/125 × 100 |
|---|---|---|---|---|---|---|---|
| Frozen reticulocyte (from | 3 | 447 (15) | 216 (33) | 54 (2) | 393 | 162 | 41 |
| Table 1) | 10 | 1143 (62) | 265 (76) | 46 (6) | 1097 | 219 | 20 |
| Frozen kidney 20 min | 3 | 567 (22) | 206 (20) | 108 (2)* | 459 | 96 | 21 |
| preincubation + frozen | 10 | 766 (15) | 190 (6) | 104 (5)* | 662 | 86 | 13 |
| reticulocyte |  |  |  |  |  |  |  |
| Frozen reticulocyte + 0.1 | 3 | 97 (25) | 83 (21) |  |  |  |  |
| mM carrier alanine | 10 | 102 (7) | 74 (10) |  |  |  |  |
| Frozen kidney + 0.1 mM | 3 | 97 (9) |  |  |  |  |  |
| carrier alanine | 10 | 105 (19) |  |  |  |  |  |
| Frozen reticulocyte + 19 | 3 | 455 (13) | 211 (19) |  |  |  |  |
| other amino acids, 0.1 | 10 | 964 (77) | 221 (4) |  |  |  |  |
| mM each |  |  |  |  |  |  |  |

The influence of preincubation and the addition of alanine or other 19 amino acid carriers on the incorporation of $H^3$-alanine (1 × 10$^{-7}$ *M*) into sRNA fractions 125 and 150. The concentrations of each enzyme and other components are the same as in Table 1. In the tubes of line two, kidney enzyme, sRNA, and buffer were pre-incubated 20 min at 28°C before the $H^3$-alanine, ATP, and reticulocyte enzyme were added. The values represent averages of duplicate incubations. Numbers in parentheses represent one half of the range.
\* The blank did not receive a preincubation period.

Lines 3, 4, and 5 of Table 2 demonstrate that the counts incorporated into sRNA's 125 and 150 by reticulocyte enzyme and into sRNA 125 by kidney enzyme do, in fact, represent largely alanine, since only alanine (of the 20 carrier amino acids added) appreciably reduced the counts incorporated.

*Discussion.*—The four principal findings presented above are consistent with a codon-restriction model of differentiation and aging. Indeed, whereas other mechanisms of cell differentiation may also exist, it is difficult to see how, within the confines of present molecular genetic concepts, rabbit kidney could synthesize appreciable quantities of proteins containing alanine decoded exclusively by the alanyl sRNA(s?) present in fraction 150. Whether or not the model is generally relevant will ultimately be determined by the presence or absence of sufficient differences between tissues of other activating enzymes or of sRNA's to permit organisms to utilize the flexibility in synthetic pattern and control implicit in such a model.

Although the quantitative differences here reported between liver and reticulo-cytes in "peak III" leucine-activating capacity are substantial (Fig. 3), the signifi-cance of this difference is unclear. Possibly immature reticulocytes possess a full complement of synthetases, whereas more fully differentiated cells have completely lost "peak III"-specific synthetases. A mixture of two such cell types could account for the fact that the ratios of counts incorporated into peak III material by reticulocytes is only about half of the value observed in the region of peaks I and II.

More direct evidence bearing on the hypothesis of cell specialization as a result of the absence of sRNA-specific synthetases is given by the experiments in which the specificities of kidney synthetases were compared with those of liver or reticu-locytes. The virtual absence of peak-150-specific charging capacity in kidney preparations strongly suggests that this enzyme is absent in the tissue of origin. Both quantitative and qualitative differences among tissues could have arisen through inadvertent fractionation during extraction or in the course of the removal of carrier amino acid and endogenous sRNA's through treatment with Sephadex G-100. Although this possibility has not been ruled out, it appears to be unlikely; for the results obtained would require that similar aminoacyl-sRNA synthetases

(in terms of their sRNA specificity but present in different tissues) have grossly different properties. In this, as in other analogous studies, the chromatographic separation of synthetase-specific sRNA's for a given amino acid does not establish that these sRNA fractions decode different triplets *in vivo*.

At the time these studies were initiated, the evidence available from studies on microorganisms appeared to rule out the hypothesis within such organisms. For example, Sueoka has presented evidence that a single enzyme is involved in the attachment of leucine to two species of *Escherichia coli* sRNA.[17] Nevertheless, it was not clear to us, *a priori*, that bacteria are an appropriate model for metazoan differentiation, for metazoa may well utilize potentialities resident within codon specificities that are of little selective advantage to an undifferentiating or primitive form which does not usually undergo multiple and substantial degrees of cellular specialization.

A recent report by Barnett and Epler[18] of the presence of several pairs of sRNA-specific enzymes in *Neurospora* (phenylalanine and aspartic acid sRNA synthetases) is consistent with the results reported in the present communication, as is also the finding by Yu[19] that even in *E. coli* different leucyl-sRNA species are activated by different protein fractions.

The potentialities for differential genomic expression implicit in the selective codon activation (or restriction) hypothesis are probably sufficient to account for the diversity of cell types and function that occurs in metazoans. For example, if each amino acid were represented in a synthetic set by only one codon out of an average of about three available, the number of different sets possible would be about $3^{20}$ or $\sim 10^8$. If one set of codons, perhaps a basic set common to all cells of an organism, were removed as a source of variation, the remaining 20 pairs would still permit $2^{20} \sim 10^6$ different distinguishable cell-specific synthetic groups to be encoded. Combinations of two or more sets per cell type would further increase the number of possibilities. It should be pointed out, however, that the activation of more than one codon set per cell type poses restrictions because certain combinations of sets are equivalent to certain other combinations.[7] Also of potential relevance are recent studies which indicate that single species of bacterial sRNA can attach to more than one kind of triplet messenger for a given amino acid.[20, 21]

The model of cell differentiation and control which prompted these experiments, while based on the interplay of fewer than 64 similar variables (aminoacyl-sRNA synthetases), also imposes certain coding rigidities. For example, if nucleotide substitution (mutation) in the coding of cell-specific protein occurs, such a substitution must be restricted only to those possibilities which yield triplets that are still within the codon set(s) translatable by the cell in question; otherwise, the mutated message would become untranslatable within the cell and the product would not be produced.

Differentiation is usually associated with a decrease in or loss of capacity for growth and cell division.[22, 23] This general antagonism suggests that suppression of growth results from the repression or loss of those synthetic capacities characteristic of cell growth and division as differentiation and specialization take place. If repression, perhaps as outlined above, occurs via the loss of specific aminoacylated-sRNA species, it is apparent that all those syntheses, specialized or not, that employ a repressed triplet will also be repressed. Finally, if during development any of

these suppressed syntheses gave rise to long-lived components essential to function, components which deteriorate slowly and which cannot be replaced after maturation, it is obvious that senescence will be an ultimate consequence of such differential repression. It is also clear that derepression by humoral factors,[24] by viral agents,[25] or by mutation could produce reversions to earlier (ontogenetically) patterns of synthesis, characteristic of controlled or uncontrolled growth, and that pharmacological modification of the pattern and tempo of codon set translation is not beyond possibility.

[1] Weismann, A., *Essays Upon Heredity and Kindred Biology Problems* (London: Oxford Univ. Press [Clarenden], 1891).

[2] Minot, C. S., *Popular Sci. Monthly,* **71** (June), 481–496: (Aug.), 97–120; (Dec.), 509–523 (1907).

[3] Pearl, R., *Sci. Monthly,* **12,** (March–Sept.), 193–212 (1921).

[4] Medawar, P. B., *An Unsolved Problem of Biology,* Inaugural lecture, University College, London (London: Lewis, 1951).

[5] Bonner, J., *Molecular Biology of Development* (Oxford: Oxford University Press, 1965).

[6] Allfrey, V. G., and A. E. Mirsky, in *Cold Spring Harbor Symposia on Quantitative Biology,* vol. 28 (1963), pp. 247–262.

[7] Strehler, B. L., *Code Degeneracy and the Aging Process: A Molecular-Genetic Theory of Aging,* 7th International Congress of Gerontology, Vienna, 1966, pp. 177–185; Strehler, B. L., *Cellular Aging: Fact and Hypothesis,* Society for Experimental Biology, Symposium on Aging, Sheffield, England, 1966, in press.

[8] Stent, G. S., *Science,* **144,** 816 (1964).

[9] Itano, H., Proceedings of the Symposium on Abnormal Haemoglobins, Ibadan, Nigeria, 1963.

[10] Ames, B. N., and P. Hartman, in *Cold Spring Harbor Symposium on Quantitative Biology,* vol. 28 (1963), p. 349.

[11] Keller, E. B., and P. C. Zamecnik, *J. Biol. Chem.,* **221,** 45 (1956).

[12] Borsook, H., C. L. Deasy, A. J. Haagen-Smit, G. Keighley, and P. H. Lowy, *J. Biol. Chem.,* **196,** 669 (1952).

[13] Lamborg, M. R., and P. C. Zamecnik, *Biochemistry,* **4,** 63 (1965).

[14] Yamane, T., and N. Sueoka, these PROCEEDINGS, **50,** 1093 (1963).

[15] Bruno, G. A., and J. E. Christian, *Anal. Chem.,* **33,** 1916 (1961).

[16] Kelmers, A. D., G. D. Novelli, and M. P. Stulberg, *J. Biol. Chem.,* **240,** 3979 (1965).

[17] Yamane, T., and N. Sueoka, these PROCEEDINGS, **51,** 1178 (1964).

[18] Barnett, W. E., and J. L. Epler, these PROCEEDINGS, **55,** 184 (1966).

[19] Yu, C. T., in *Cold Spring Harbor Symposium on Quantitative Biology,* vol. 31 (1966).

[20] Crick, F. H. C., *J. Mol. Biol.,* **19,** 548 (1966).

[21] Kellogg, D. A., B. P. Doctor, J. E. Loebel, and M. W. Nirenberg, these PROCEEDINGS, **55,** 912 (1966).

[22] Konigsberg, I. R., *Newsletter (Geront. Soc.),* **7** (3), 33–34 (1960).

[23] Strehler, B. L., I. R. Konigsberg, and J. E. T. Kelley, *Exptl. Cell Res.,* **32,** 232 (1962).

[24] Clever, U., *Chromosoma,* **12,** 607 (1961).

[25] Kano-Sueoka, T., and N. Sueoka, *J. Mol. Biol.,* **20,** 183 (1966).

[26] Allen, E. H., and R. S. Schweet, *J. Biol. Chem.,* **237,** 760 (1962).