[8] Felsenfeld, G., and S. Huang, *Biochim. Biophys. Acta*, **51**, 19 (1961).

[9] Lyons, J. W., and L. Kotin, *J. Am. Chem. Soc.*, **86**, 3634 (1964).

[10] Boedtker, H., *J. Mol. Biol.*, **2**, 171 (1960).

[11] Monier, R., and M. Grunberg-Manago, in *Acides Ribonucléiques et Polyphosphates*, Colloques Internationaux de Centre National du la Recherche Scientifique no. 106 (Paris, 1962), p. 163.

[12] Giacomoni, D., and S. Spiegelman, *Science*, **138**, 1328 (1962).

[13] Mahler, H. R., G. Dutton, and H. Mehrotra, *Biochim. Biophys. Acta*, **68**, 199 (1963).

[14] Nishimura, S., and G. D. Novelli, *Biochem. Biophys. Res. Commun.*, **11**, 161 (1963).

[15] Nishimura, S., and G. D. Novelli, *Biochim. Biophys. Acta*, **80**, 574 (1964).

[16] Penniston, J. T., and P. Doty, *Biopolymers*, **1**, 145 (1963).

[17] Allende, J. E., C. C. Allende, M. Gatica, and M. Matamala, *Biochem. Biophys. Res. Commun.*, **16**, 342 (1964).

[18] Norris, A., and P. Berg, these PROCEEDINGS, **52**, 330 (1964).

[19] Lagerkvist, U., and J. Waldenström, *J. Biol. Chem.*, **240**, Pc 2264 (1965).

[20] Allende, J. E., G. Mora, M. Gatica, and C. C. Allende, *J. Biol. Chem.*, **240**, Pc 3229 (1965).

[21] Cf. P. Berg, *Ann. Rev. Biochem.*, **30**, 293 (1961).

[22] Holley, R. W., *Biochem. Biophys. Res. Commun.*, **10**, 186 (1963).

[23] Lindahl, T., and J. R. Fresco, in *Methods in Nucleic Acids*, ed. L. Grossman and K. Moldave (New York: Academic Press, 1966), vol. 1, in press.

[24] Lindahl, T., D. Henley, and J. R. Fresco, *J. Am. Chem. Soc.*, **87**, 4961 (1965).

[25] Tissières, A., *J. Mol. Biol.*, **1**, 365 (1959).

[26] Loftfield, R. B., and E. A. Eigner, *Acta Chem. Scand.*, **17**, S117, (1963).

[27] Holley, R. W., J. Apgar, G. A. Everett, J. T. Madison, S. H. Merrill, and A. Zamir, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 117.

[28] Apgar, J., R. W. Holley, and S. H. Merrill, *J. Biol. Chem.*, **237**, 796 (1962).

[29] Möller, W., and H. Boedtker, in *Acides Ribonucléiques et Polyphosphates*, Colloques Internationaux de Centre National du la Recherche Scientifique no. 106 (Paris, 1962), p. 99.

[30] The presence of several sRNA components for both leucine and arginine in *yeast* has been described earlier. Thiebe and Zachau observed two leucyl- and three arginyl-sRNA's after countercurrent distribution in a solvent system different from that used here [Thiebe, R., and H. G. Zachau, *Biochim. Biophys. Acta*, **103**, 568 (1965)]. Cherayil and Bock found three distinct peaks for leucyl acceptor activity by chromatography on DEAE columns in the presence of urea [Cherayil, J. D., and R. M. Bock, *Biochemistry*, **4**, 1174 (1965)]. Sequence analysis on C^{14}-leucyl-oligonucleotides obtained after enzymatic digestion of aminoacyl-sRNA gave evidence for the presence of three (or more) different leucyl sRNA's (Herbert, E., personal communication).

[31] Our colleagues at this University, W. Gartland and N. Sueoka, have independently made an apparently related observation. See the adjacent paper, these PROCEEDINGS, **55**, 948 (1966).

[32] Fuwa, K., W. E. C. Wacker, R. Druyan, A. Bartholomay, and B. L. Vallee, these PROCEEDINGS, **46**, 1298 (1960).

---

*TWO INTERCONVERTIBLE FORMS OF TRYPTOPHANYL SRNA IN E. COLI**

By WILLIAM J. GARTLAND† AND NOBORU SUEOKA

DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

Communicated by Colin S. Pittendrigh, February 28, 1966

Involvement of sRNA as the adaptor between an RNA template and a polypeptide chain[1, 2] predicts that this class of molecules should have at least three functional sites. One site confers specificity for recognition by the correct aminoacyl-sRNA synthetase, another serves as an anticodon for hydrogen bonding to specific

codon triplets on the template, and a third functions as a site for binding to the ribosome. None of these sites has been clearly established. The primary structure of yeast alanyl sRNA as determined by Holley *et al.* suggests several possible secondary structures.[3] The possibility exists that more than one configuration is stable in the same condition. These alternative configurations may exhibit some functional differences.

In this report, we present a case in which *E. coli* tryptophanyl sRNA can exist in two alternative forms (I and II), both of which are stable in neutral buffer with the presence of Mg^{++}. Interconversion can be achieved without discharging tryptophan. Conversion from form I to form II can be achieved in relatively mild conditions, i.e., neutral pH in the absence of magnesium. Regeneration of form I requires exposure of form II to low pH or water dialysis. It was found that *E. coli* tryptophanyl sRNA synthetase can recognize only one (form I) of the two forms. Codon recognition of the two forms is also different and will be reported elsewhere.[4]

Materials and Methods.—Preparation of sRNA: sRNA was prepared from log phase *E. coli* B by the phenol procedure described by von Ehrenstein and Lipmann,[5] or obtained commercially from General Biochemicals. The latter material had been stripped in glycine buffer at pH 8.5.

Preparation of enzyme extract: Essentially the method of Zubay[6] was used. *E. coli* B cells were harvested in log phase, frozen at −80°C, and ground with 3 times their wet weight of alumina. To the crude extract from a 1-liter culture, 10 ml of Tris-magnesium buffer (0.01 *M* Tris-HCl buffer pH 7.3, 0.001 *M* $MgCl_2$) were added, and the mixture was centrifuged at 12,000 × *g* for 10 min. The supernatant was centrifuged at 105,000 *g* for 3 hr at 4°C. The upper $^2/_3$ of the supernatant was dialyzed for 12 hr at 4°C against 4 liters of Tris-magnesium buffer containing 0.006 *M* mercaptoethanol. Enzyme extract was stored at −80°C.

Preparation of tryptophanyl sRNA: Tryptophanyl sRNA was prepared basically according to Berg *et al.*[7] The reaction mixture consisted of the following: 0.1 *M* Tris-HCl buffer (pH 7.3), 4.8×10^{-3} *M* magnesium acetate, 4×10^{-3} *M* reduced glutathione, 3–8 mg sRNA, 9 μc H^3-tryptophan (3000 μc/μmole, Schwarz BioResearch, Inc.), approximately 20 μg enzyme protein, and ATP as indicated. Total reaction vol was 2.5 ml. The reaction mixture was incubated for 20 min at 37°C.

Charged sRNA was normally isolated from the reaction mixture by shaking for 5 min at room temperature with 2 vol of redistilled water-saturated phenol (Mallinckrodt). The aqueous phase was extracted twice with ether and the sRNA precipitated at −15°C with 2 vol of ethanol.

Conditions for enzymatic discharge: Enzymatic discharge was carried out under conditions similar to those of Berg *et al.*[7] The discharge mixture contained: 0.1 *M* Tris-HCl buffer (pH 6.9), 4.8×10^{-3} *M* magnesium acetate, 4×10^{-3} *M* AMP, 4×10^{-3} *M* pyrophosphate, and approximately 20 μg enzyme protein in a total vol of 2.5 ml. Incubation was carried out at 37°C. Aliquots (0.2 ml) were withdrawn as a function of time, precipitated in 2 ml cold 10% TCA, and collected on membrane filters. The filters were dried and placed in glass vials filled with 15 ml toluene containing 1.5 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 60 mg of 2,5-diphenyloxazole (PPO). Counting was carried out in a Packard Tricarb liquid scintillation counter with a double counting efficiency of 6% for H^3, 34% for C^{14}.

Fractionation of sRNA on methylated albumin column: sRNA charged with radioactive tryptophan was fractionated on methylated albumin kieselguhr (MAK) columns as previously described,[8] except that a linear gradient was established between 110 ml 0.35 *M* sodium chloride and 110 ml 1.2 *M* sodium chloride in 0.05 *M* sodium phosphate buffer, pH 6.7. Two-ml fractions were precipitated in the presence of carrier DNA with 2 vol of cold 10% TCA, collected on membrane filters, and counted.

Results.—The MAK column elution patterns of tryptophanyl sRNA show marked differences depending on the concentration of ATP present in the charging mixture. When sRNA is charged in the presence of 0.002 *M* ATP and isolated

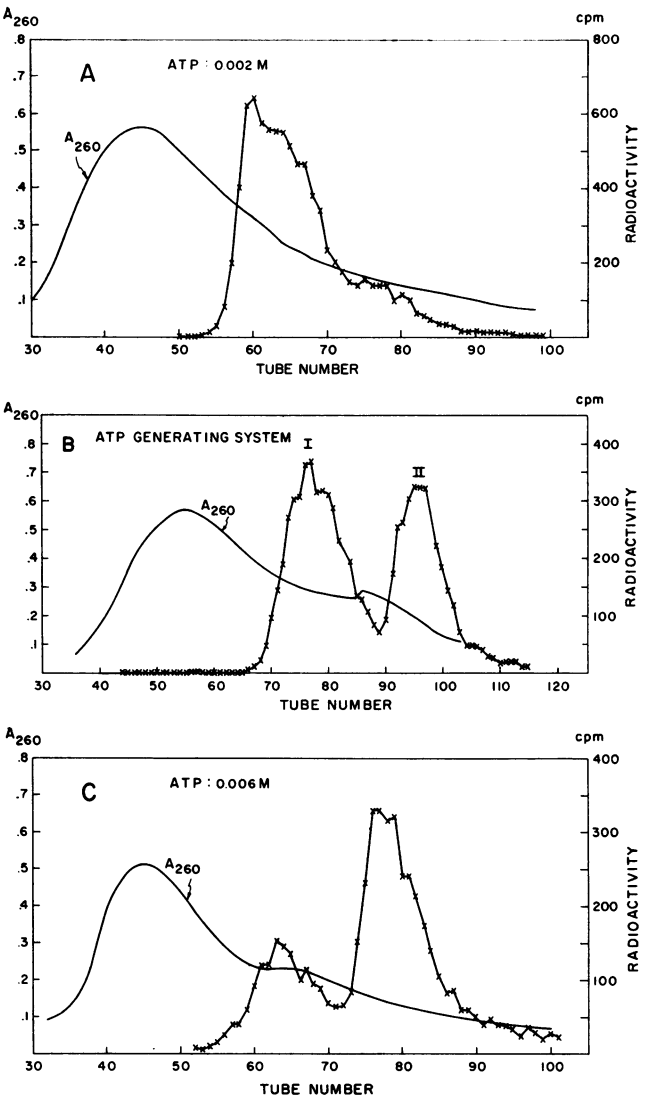

FIG. 1.—Elution patterns of *E. coli* H^3-tryptophanyl sRNA prepared by charging sRNA in the presence of different amounts of ATP and isolated as described in *Materials and Methods*. (*A*) H^3-tryptophanyl sRNA charged in 0.002 *M* ATP; (*B*) H^3-tryptophanyl sRNA charged in 0.002 *M* ATP plus an ATP generating system consisting of 0.002 *M* PEP and 100 μg phosphoenolpyruvate kinase; (*C*) H^3-tryptophanyl sRNA charged in 0.006 *M* ATP.

as described under *Materials and Methods*, tryptophanyl sRNA elutes as a single peak (form I) which appears approximately 15 tubes after the optical density peak (Fig. 1*A*). If an ATP generating system consisting of 0.002 *M* phosphoenolpyruvate and 100 μg phosphoenolpyruvate kinase is added to the reaction mixture containing 0.002 *M* ATP, the MAK profile of tryptophanyl sRNA consists of two approximately equal components (Fig. 1*B*). The second peak of tryptophanyl-sRNA (form II) elutes nearly 20 tubes later than form I. When charging is carried

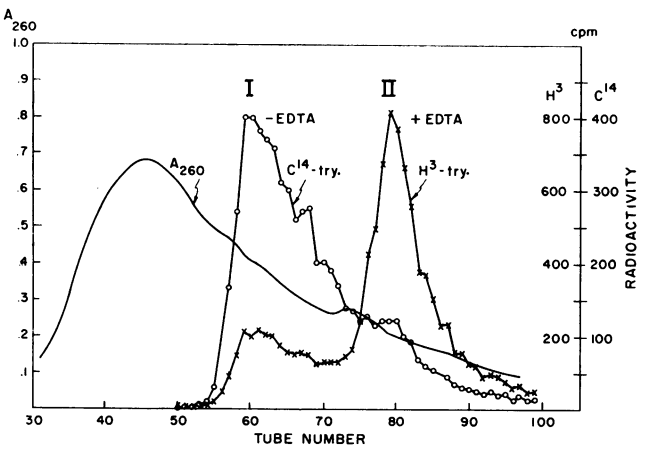

FIG. 2.—Comparison of tryptophanyl sRNA elution patterns with and without EDTA treatment. C^{14}-try sRNA was charged in 0.002 *M* ATP and isolated as described in *Materials and Methods*. H^3-try sRNA was charged in 0.002 *M* ATP for 20 min, and then EDTA added to the reaction mixture to 0.004 *M* just prior to phenol shaking.

out in a reaction mixture containing 0.006 *M* ATP, form II predominates (Fig. 1*C*).

To determine whether high ATP concentration was specifically required during attachment of tryptophan to sRNA to cause conversion of form I to form II, the following experiments were carried out. sRNA was charged under conditions producing only form I (0.002 *M* ATP). After 20 min of charging, 10 μmoles of ATP were added, raising the final concentration of ATP to at least 0.004 *M*, and the reaction mixture was immediately shaken with phenol. This procedure was found to cause conversion of the majority of tryptophanyl sRNA to form II. Addition of GTP or CTP to 0.004 *M* after 20 min of charging in 0.002 *M* ATP, followed by phenol shaking, produced a similar conversion to form II, whereas addition of AMP to 0.004 *M* results in no conversion to form II.

The similar results obtained with different nucleotide triphosphates led us to investigate the role of a chelating agent in this phenomenon. The experiment is similar to those described above. sRNA was charged with tryptophan in a reaction mixture containing 0.002 *M* ATP. After 20 min the reaction mixture was brought to 0.004 *M* EDTA and shaken with phenol. This procedure causes a rapid conversion and is routinely used in the preparation of form II. Under these conditions we frequently observe a 10 per cent or less contamination of the form II preparation with form I. A similar result is obtained if shaking with phenol is omitted, and the reaction mixture is incubated in 0.004 *M* EDTA for 5 min followed by ethanol precipitation. Simultaneous elution of form I (C^{14}) and form II (H^3) on the same MAK column indicates that both forms are stable in the eluting buffer at 24°C and fractionate independently (Fig. 2).

Stability and interconversion of the two forms: In view of the apparent lability of form I under charging conditions in the absence of Mg^{++}, we decided to investigate the phenomenon in simplified buffer systems. Form I is unstable and converts to form II when incubated for 20 min in 0.1 *M* sodium acetate buffer pH 5.6 or 0.1 *M* sodium phosphate buffer pH 7.0 in the absence of Mg^{++}. Form I, however, is found

TABLE 1

CONDITIONS FOR INTERCONVERSION AND STABILITY OF TRYPTOPHANYL sRNA I AND II

*Conditions converting form I to form II**
(1) 0.1 *M* Sodium acetate buffer pH 5.6
(2) 0.1 *M* Sodium phosphate buffer pH 7.0

Conditions converting form II to form I
(1) 0.1 *M* Sodium acetate buffer pH 4.1 or pH 3.5*
(2) Dialysis against distilled H_2O, 12 hr, 4°C

Conditions stabilizing both forms I and II
(1) sRNA charging reaction mixture†
(2) MAK eluting buffer: 0.35–1.2 *M* NaCl in 0.05 *M* sodium phosphate buffer, pH 6.7, 24°C
(3) 0.1 *M* Sodium phosphate buffer pH 7.0 containing 0.02 *M* magnesium acetate*
(4) Conditions for binding sRNA to ribosomes:‡
0.05 *M* Tris-acetate buffer pH 7.2
0.05 *M* Potassium acetate
0.02 *M* Magnesium acetate

* Conditions for buffer incubations: Tryptophanyl sRNA I or II was incubated at 37°C in the indicated buffer. After 20 min the buffers were diluted into 20 vol 0.35 *M* NaCl, 0.05 *M* sodium phosphate buffer pH 6.7, 10°C, and profiled on MAK column.
† 0.1 *M* Tris-HCl, 4.8×10^{-3} *M* MgAc, 4×10^{-3} *M* reduced glutathione, 2×10^{-3} *M* ATP, 20 min, 37°C.
‡ Incubation for 15 min at 24°C.

to be stable to 0.1 *M* sodium phosphate buffer pH 7.0 containing 0.02 *M* magnesium acetate. Form I is stable in MAK starting buffer (0.35 *M* NaCl, 0.05 *M* sodium phosphate buffer, pH 6.7) at 24°C, but incubation at 37°C for 20 min causes conversion to form II.

Form I can be regenerated from form II by incubation of the latter in 0.1 *M* sodium acetate buffer pH 3.5, or by dialysis against distilled water for 12 hr at 4°C. Form II is stable to a 20-min incubation in the charging mixture which contains 4.8×10^{-3} *M* magnesium acetate. However, phenol shaking in the latter condition converts form II to form I. Form II suspended in 0.1 *M* sodium phosphate buffer pH 7.0 containing 0.02 *M* magnesium acetate shows at most a 20 per cent back conversion to form I after a 20-min incubation at 37°C. Both forms are stable in the reaction mixture used for studying the binding of sRNA to ribosomes.[9] Results are summarized in Table 1.

Enzyme recognition of the two forms: To determine the affinity of tryptophanyl sRNA synthetase for the two forms of tryptophanyl sRNA, enzymatic removal and attachment of tryptophan to sRNA were investigated. Form I was obtained by charging tryptophanyl sRNA in low ATP. Form II was obtained from form I by phenol shaking in the presence of EDTA.

When aminoacyl sRNA is incubated in the presence of enzyme, excess AMP, and pyrophosphate (see *Materials and Methods*), the esterified amino acid is normally removed. Tryptophanyl sRNA form I shows a rapid loss of tryptophan under the enzymatic discharge conditions. The sRNA is stripped of approximately 75 per cent of initial tryptophan counts within 2 min. Tryptophanyl sRNA form II, however, shows an initial loss of counts, and then levels off at a value representing approximately 20 per cent discharge (Fig. 3). Both forms show little loss of counts in minus enzyme control indicating negligible nonenzymatic hydrolysis. The initial enzymatic loss of counts from form II is probably due to the presence of contaminating form I in the preparation.

Another experiment designed to study enzyme recognition of the two forms is to examine attachment of tryptophan. sRNA was first charged with H^3-tryptophan in low ATP. One half of this form I preparation was treated with EDTA

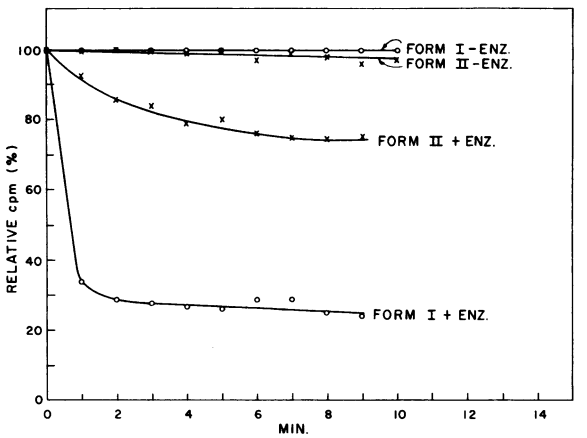

FIG. 3.—Enzymatic detachment of H^3-tryptophan from tryptophanyl sRNA. Forms I and II were incubated for the indicated time, and the amount of tryptophan bound to sRNA was determined by the amount of acid-precipitable H^3 counts. Complete incubation mixtures contained enzyme, AMP, and pyrophosphate.

and phenol to produce form II. These two sRNA preparations were then exposed to enzyme in separate charging mixtures containing 3 μc C^{14}-tryptophan (24 μc/μmole). The reaction mixtures were incubated for 20 min at 37°C followed by precipitation of the sRNA by 2 vol of ethanol at −15°C. Precipitated sRNA samples were resuspended in starting buffer and profiled. Phenol shaking was omitted in the procedure because form II was known to be unstable to phenol shaking in the reaction mixture in the absence of EDTA and converted to form I. Both forms are stable to ethanol precipitation, however. Results show that form I (Fig. 4*A*) was able to exchange H^3-tryptophan for C^{14}-tryptophan. Form II (Fig. 4*B*), however, showed no isotope exchange, and the small incorporation of C^{14}-tryptophan was located in the residue of form I.

Other soluble RNA's: We have examined 11 other aminoacyl sRNA's to determine whether they undergo a similar conversion. In each case a C^{14} amino acid was charged to sRNA in 0.002 *M* ATP. The H^3 amino acid was charged under similar conditions, except that the reaction mixture was brought to 0.004 *M* EDTA just prior to shaking with phenol. In each case both isotopes were profiled on the same MAK column. The following aminoacyl sRNA's were tested: alanine, methionine, phenylalanine, valine, tyrosine, leucine, isoleucine, arginine, serine, proline, and lysine. No difference in profile could be detected for these aminoacyl sRNA's.

Discussion.—The present experiments demonstrate that tryptophanyl sRNA can exist in two distinct conformations which are separable on the methylated albumin column. Both forms are stable at neutral pH in the presence of magnesium.

Removal of magnesium at neutral pH with moderate ionic strength (Table 1) converts tryptophanyl sRNA I to II. The tryptophanyl sRNA II thus obtained is stable. Regeneration of tryptophanyl sRNA I is accomplished by exposing tryptophanyl sRNA II to conditions which weaken hydrogen-bonded structures (low pH, water dialysis) followed by restabilization with magnesium or low temper-

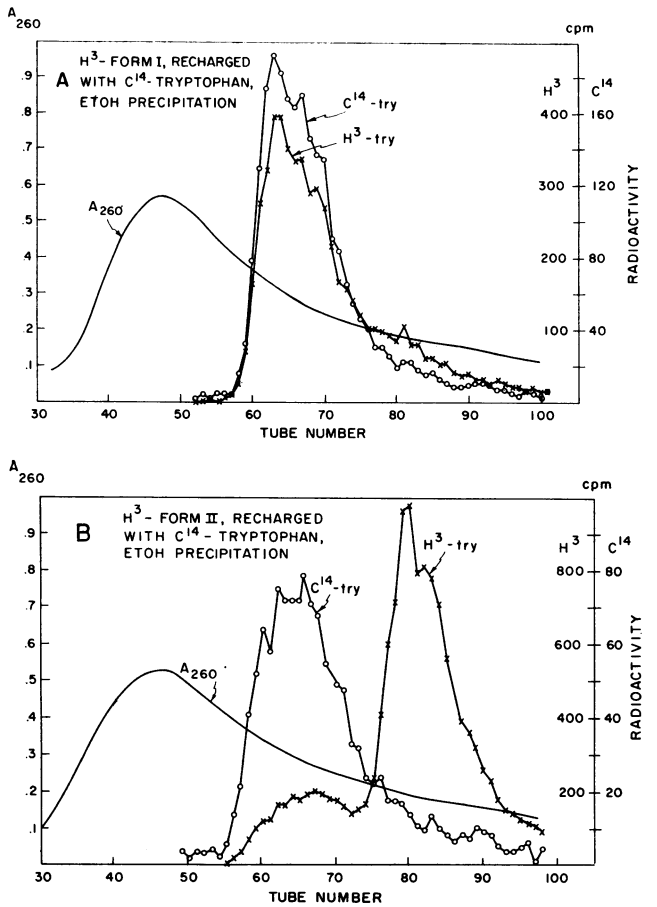

FIG. 4.—Chargeability of tryptophanyl sRNA forms I and II. (*A*) H^3-tryptophanyl sRNA form I incubated in the charging mixture with enzyme and 3 μc C^{14}-try. (*B*) H^3-tryptophanyl sRNA form II incubated in the charging mixture with enzyme and 3 μc C^{14}-try. After 20 min incubation, sRNA was precipitated with 2 vol of ethanol at −15°C, resuspended in starting buffer, and profiled.

ature. It is difficult at this time to conclude whether magnesium is stabilizing the secondary structure of tryptophanyl sRNA, or whether it is absolutely necessary as an integral part of the molecule.[15]

The nature of the structural change accompanying the interconversion cannot be defined yet. The following possibilities are raised:

Slippage: The configurational difference between the two may be the result of rearrangement of the base pairing pattern with an alteration of the secondary structure. The over-all degree of hydrogen bonding would not have to be widely different in the two configurations.

Aggregation: Form II could represent an aggregate (e.g., dimer). If so, the aggregation should be a specific one, because of the clear-cut separation of the two forms. Apparent aggregation of sRNA chains has been reported.[16]

Partial denaturation: During destabilization and conversion of try I to try II,

a portion of the molecule might be denatured and not readily renaturable. Such a partially denatured structure would be expected to elute at higher salt concentrations on the MAK column.

At our present state of knowledge, we are not capable of distinguishing among these three alternatives. However, the first possibility is most attractive, since it provides reasonable support for our finding in the change of functional aspects of the molecule: loss of enzyme recognition (Figs. 3 and 4) and alteration of codon recognition. Methylation[10-12] or oxidation of thiopyrimidine[13, 14] for the interconversion is unlikely under the conditions used in the present experiment.

A residue of form I, usually about 10 per cent, is observed in our form II preparations. This could be due to incomplete conversion to form II, or represent a minor class of tryptophanyl sRNA molecules incapable of undergoing the transition.

The *in vivo* significance of the interconversion of the tryptophanyl sRNA cannot yet be evaluated. The fact that one configuration is chargeable, and the other not, could be of significance if both configurations exist *in vivo*. The two configurations are interesting from the standpoint of the polypeptide-polynucleotide interaction which provides specificity for the amino acid activating enzymes.

Various ratios of the two tryptophanyl-sRNA peaks on the MAK column observed in our earlier studies can now be explained by the interconversion. Countercurrent distribution of *E. coli* tryptophanyl sRNA would not be capable of detecting form II, because of its inability to accept tryptophan enzymatically. The present phenomenon should be taken into consideration in studies on the codon assignment of tryptophan in *E. coli*.

Tryptophan sRNA is the only sRNA of 12 examined which shows a detectable change of MAK column profile when subjected to the EDTA, phenol treatment.

Summary.—Tryptophan sRNA exists in two distinct configurations which are resolvable by MAK chromatography. The interconversions are reversible, nonenzymatic, and can be elicited by several distinctly different treatments. Tryptophanyl sRNA synthetase has affinity for only one of the configurations.

* This work was supported by NSF grant GB 3445 and NIH grant GM 10923.

† Predoctoral trainee in biochemical sciences under NIH graduate training grant 5TI GM 962.

[1] Crick, F. H. C., in *The Structure of Nucleic Acids and Their Role in Protein Synthesis* (Cambridge University Press, 1957), p. 25.

[2] See reviews by Crick, F. H. C., in *Progress in Nucleic Acid Research* (Academic Press, 1963), vol. 1, p. 163: Sueoka, N., and T. Yamane, in *Symposium on Informational Macromolecules* (Academic Press, 1963), p. 205.

[3] Holley, R., J. Apgar, G. Everett, J. Madison, M. Marquisee, S. Merrill, J. Penswick, and A. Zamir, *Science*, **147**, 3664 (1965).

[4] Gartland, W., N. Sueoka, and M. Nirenberg, in preparation.

[5] von Ehrenstein, G., and F. Lipmann, these PROCEEDINGS, **47**, 941 (1961).

[6] Zubay, G., *J. Mol. Biol.*, **4**, 347 (1962).

[7] Berg, P., F. H. Bergmann, E. J. Ofengand, and M. Dieckmann, *J. Biol. Chem.*, **236**, 1726 (1961).

[8] Sueoka, N., and T. Yamane, these PROCEEDINGS, **48**, 1454 (1962); Yamane, T., and N. Sueoka, these PROCEEDINGS, **50**, 1093 (1963).

[9] Brimacombe, R., J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, and T. Jaouni, these PROCEEDINGS, **54**, 954 (1965).

[10] Fleissner, E., and E. Borek, these PROCEEDINGS, **48**, 7 (1962).

[11] Hurwitz, J., M. Gold, and M. Anders, *J. Biol. Chem.*, **239**, 10 (1964).

[12] Peterkofsky, A., these PROCEEDINGS, **52**, 5 (1964).

[13] Carbon, J., L. Hung, and D. Jones, these PROCEEDINGS, **53**, 979 (1965).

[14] Lipsett, M., *Biochem. Biophys. Res. Commun.*, **20**, 224 (1965).

[15] An apparently related phenomenon has been observed at Princeton by Lindahl, Adams, and Fresco, and is reported in the adjacent paper (941–948).

[16] Schleich, T. and, J. Goldstein, these PROCEEDINGS, **52**, 744 (1964); Röschenthaler, R., and P. Fromageot, *J. Mol. Biol.*, **11**, 458 (1965).

---

*INDUCTION OF CELLULAR DNA SYNTHESIS BY POLYOMA VIRUS, III. INDUCTION IN PRODUCTIVELY INFECTED CELLS**

BY MARGUERITE VOGT, RENATO DULBECCO, AND BASIL SMITH†

THE SALK INSTITUTE FOR BIOLOGICAL STUDIES, SAN DIEGO, CALIFORNIA

Communicated February 18, 1966

In cultures of mouse kidney cells, polyoma virus causes a productive infection, which is characterized by viral multiplication and leads to cell death. In crowded cultures, in which there is little DNA synthesis, the virus causes a stimulation of the synthesis of cellular DNA[1–3] and of enzymes involved in DNA synthesis.[4, 5] A similar induction is produced by simian virus 40,[6] but not by viruses that do not produce tumors, hence its considerable interest as a possible factor in cell transformation caused by these viruses.

Induction of cellular DNA synthesis by polyoma virus had so far been observed under conditions where not all cells were productively infected. In contrast with these findings, Sheinin and Quinn[7] showed that in uncrowded mouse embryo cell cultures, in which practically all the cells are productively infected, induction does not occur and cellular DNA synthesis is inhibited. The suspicion thus arises that the induction occurs in cells that are not productively infected, i.e., either in abortively infected cells or, as an indirect phenomenon, in uninfected cells.

In order to clarify the significance of the induction of cellular DNA synthesis, confluent coverslip cultures of mouse kidney cells were infected with polyoma virus, and pulse-labeled with H^3-thymidine at a time at which a high proportion of the DNA synthesized was cellular. The cultures were fixed after an appropriate time of incubation to allow for the synthesis of the viral capsid protein. A combined study of the same cells by radioautography and immunofluorescence showed that at least 90 per cent of the cells synthesizing DNA at the time of the pulse had also made viral capsid antigen at the time of fixation. These results conclusively showed that the induction of cellular DNA synthesis takes place in productively infected cells.

Materials and Methods.—Virus and cell cultures: Polyoma virus of the large plaque type and primary cultures of mouse kidney cells were prepared as described previously.[1] The cells were grown on glass coverslips, 22 × 40 mm in size, inside 60-mm plastic Petri dishes. The coverslips had been boiled previously in medium supplemented with serum to allow a better spreading of the epithelial cells.

Infection of cultures: The coverslip cultures were infected with 0.4 ml of a suspension of purified virus at the appropriate dilution. After 1 hr of incubation at 37°C, the coverslip cultures were washed three times with medium and subsequently incubated at 37°C in a well-humidified incubator flushed with a CO_2-air mixture. All control coverslip cultures were mock-infected under identical conditions using medium instead of virus.