was supported by USPHS research grant AM-03412 from the National Institute of Arthritis and Metabolic Diseases, and by postdoctoral fellowship 5-F2-GM-13,156 (to M. P. Printz) and Research Career Program Award 2-GM-K3-5479 (to P. H. von Hippel) from the National Institute of General Medical Sciences.

[1] Bradbury, E. M., W. C. Price, and G. R. Wilkinson, *J. Mol. Biol.*, **4**, 39 (1962).
[2] von Hippel, P. H., and G. Felsenfeld, *Biochemistry*, **3**, 27 (1964).
[3] Englander, S. W., *Biochemistry*, **2**, 798 (1963).
[4] Englander, S. W., and J. J. Englander, these PROCEEDINGS, **53**, 370 (1965).
[5] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
[6] Flodin, P., *J. Chromatog.*, **6**, 103 (1961).
[7] Doty, P., B. B. McGill, and S. A. Rice, these PROCEEDINGS, **44**, 432 (1958).
[8] Shack, J., *J. Biol. Chem.*, **233**, 677 (1958).
[9] Cox, R. A., and A. R. Peacocke, *J. Chem. Soc.*, 2646 (1956); 4724 (1957); 4117 (1958).
[10] Printz, M. P., and P. H. von Hippel, in preparation.
[11] Felsenfeld, G., and G. Sandeen, *J. Mol. Biol.*, **5**, 587 (1962).

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*HYDROGEN EXCHANGE STUDIES OF sRNA**

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Communicated by Walter H. Stockmayer, December 23, 1964

Following the demonstration by Printz and von Hippel[1] that the movement of exchangeable hydrogen between DNA and water could be followed at near neutral pH by use of the tritium-Sephadex method,[2] we have been studying the hydrogen exchange behavior of the various functional forms of RNA. The purpose of the present study has been to test the ability of hydrogen exchange measurements to yield information on the structure of sRNA.

Experimental.—Results to date have been obtained with four independent samples of yeast sRNA purchased from California Corp. for Biochemical Research (CBC) and from General Biochemicals, Inc. (GBI). The different preparations showed identical hydrogen exchange behavior indicating that the effect on these measurements of variable damage during preparation, of impurities, etc., is minimal. (A fifth sample from GBI was found to be aberrant and was discarded.) Preliminary hydrogen exchange experiments have also been performed with samples of serine-rich sRNA (kindly supplied by Dr. G. L. Cantoni) and with samples of valine-rich and tyrosine-rich sRNA (kindly supplied by Dr. R. W. Holley). The samples show some similarities and some differences. For present purposes we note that serine-specific sRNA which is, by Felsenfeld's dispersion of hyperchromicity analysis,[3, 4] essentially identical with mixed yeast sRNA also shows the same hydrogen exchange behavior as do the commercially obtained samples. A recently identified nontransfer RNA contaminant of 4S RNA preparations that is insoluble in cold 1 *M* NaCl[5] forms 1.6% of our current preparation (CBC).

In these experiments, exchangeable hydrogens of sRNA were initially equilibrated with tritium by brief warming (40°) in aqueous solution containing a trace of tritiated water (THO). Samples were warmed, in the absence of Mg^{++}, for periods from 10 to 30 min, and initial sRNA concentration varied from 1 to 16 mg/ml with no variation in the results. Tritiated sRNA was separated from free THO by passage through Sephadex columns washed with tritium-free buffer solutions. Methods followed were essentially those described previously.[2] Hydrogen exchange data for sRNA have also been obtained by a new technique in which separation of THO from labeled sRNA was effected by rapid dialysis. Data from the two techniques agree, indicating absence of methodological artifacts.

Some exchange-out curves are shown. Data are plotted as number of original exchangeable protons still bound to an average molecule of sRNA after various times of exchange. The parameter H/molecule is calculated from the ratio (R) of tritium activity to optical density in samples of Sephadex column effluent through the sRNA peak region. Results are computed on the basis of a 70 *nucleotide* sRNA molecule.[6] A molar extinction at 260 mμ (E_m), then, of 5.49×10^5/mole/liter/cm would be computed from reported E_P values of 7700[7, 8] and 7800.[9] However, since the sRNA samples, prepared commercially by the phenol technique of Holley *et al.*,[10] are probably missing most of the terminal adenosine, this value should be reduced by about 1 part in 71 to give 5.41×10^5/mole/liter/cm. Following reference 2, then,

$$\text{H/molecule} = 111 \times E_m \times R/C_0 = 6.00 \times 10^7 \, R/C_0,$$

in which R and E_m are defined above, 111 is the atom concentration of hydrogen in water, and C_0 is the tritium count rate in the initial equilibration mixture.

Results.—Helical content: Printz and von Hippel[1] have adduced evidence that under the conditions used here, only protons involved in hydrogen bonds exchange slowly enough to be measured. These measurements, then, can count hydrogen bonds if no equilibrium isotope effect is present. The data points plotted as filled circles in the figures represent exchange experiments in which initial sRNA-tritium (T) equilibration was accomplished in D_2O as bulk solvent, so that T competed with D for sites on sRNA. These data may be compared with the results of experiments carried out under identical conditions except that, in the initial equilibration, the competition was between T and the hydrogens of bulk solvent H_2O. Both situations led to the same degree of labeling. Thus, sRNA shows no selective binding among the hydrogen isotopes, and measurement of slowly exchanging T provides a proper index of hydrogen bonds.

At pH 6.5, in the presence of Mg^{++}, the hydrogen exchange process is so slow that only a short extrapolation from the data (Fig. 1) is necessary to obtain an estimate of the total number of hydrogen bonds in sRNA.[11] The estimate must be slightly reduced since, though time was measured in these experiments from the instant when the sRNA-THO sample entered the top of the gel column, the free THO level in the region of the sRNA band does not immediately reach zero but decreases over a finite time as the band moves downward.[12] In the present system this correction reduces the number found by simple extrapolation by 3 to give 77 hydrogen bonds per average 70 nucleotide sRNA molecule.

Mechanism of exchange: It appears that hydrogen exchange measurements can yield data sensitive to the fine structure of sRNA and also provide a measure of the contribution of some factors to its thermodynamic stability. To evaluate the data in these terms it is necessary to understand the mechanics of the hydrogen exchange process in sRNA.

In double helical nucleic acid the two hydrogen-bonded hydrogens of AU pairs and two of the three hydrogens of the GC tri are in contact with water. These could conceivably exchange with hydrogens of water by some direct chemical exchange mechanism. On the other hand, the exchange of various hydrogens might depend upon an "opening out" or "breathing" of

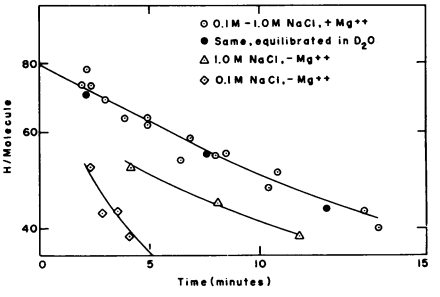

FIG. 1.—Hydrogen-tritium exchange of sRNA at pH 6.5, 4°, early times.

local structure surrounding them (involving parting of hydrogen bonds, and, presumably, some swinging out of the bases[1]).

These two mechanisms may be formulated as follows:

Mechanism 1

$$\text{closed} \xrightarrow{k_c} \text{direct chemical exchange}$$

Mechanism 2[13]

$$\text{closed} \overset{K}{\rightleftarrows} \text{open} \xrightarrow{k_3} \text{direct chemical exchange: } K = k_1/k_2$$

The rate constant k_3 of Mechanism 2 is probably close to the rate constant characteristic of fully denatured nucleic acid. This rate is too fast to measure with the present techniques. If $k_2 \gg k_3$, the intrinsic rate, k_3, will be modified by the fraction of time that the structure is open, that is, by the fraction $k_1/(k_1 + k_2) \cong k_1/k_2 = K$. (Since $T \ll T_{\text{melting}}$, $K \ll 1$, so that $k_1 \ll k_2$.) Rate of loss of tritium will then be

$$dT/dt = -Kk_3T. \tag{1}$$

The exchange curves presented show that different sites on the sRNA molecule lose bound tritium at different rates, so that equation (1) should be written

$$dT_i/dt = -K_ik_3T_i \tag{2}$$

with respect to the ith site. The exchange curves measured are generated by integration of equation (2) and summation over all sites as:

$$\sum^i T_i = \sum^i T_{0,i} \exp(-K_ik_3t). \tag{3}$$

For the case $k_2 \ll k_3$, protons will exchange at a rate equal to the rate of exposure of bases carrying them, given by $k_{1,i}$. Finally, if a hydrogen is involved in a direct chemical exchange without opening of structure (Mechanism 1), its rate constant will be $k_{c,i}$.

The effect of NaCl and of $MgCl_2$ on the exchange character of sRNA (Fig. 2) is pertinent here. Mg^{++} at low concentration (0.005–0.02 M, sufficient to neutralize the negatively charged phosphates if strong binding is assumed[17]) decreases exchange rates. Addition of NaCl through the range of concentration in which it probably affects little more than phosphate-phosphate repulsions also slows hydrogen exchange. It is difficult to imagine how these salts could act to slow reaction Mechanism 1 above. On the other hand, they are known to stabilize sRNA structure against thermal melting. In terms of Mechanism 2, these salts favor the closed form of the double helix at high temperature, and it is most likely that the reduction of exchange rates in their presence results from the same phenomenon operating at the lower temperature of these experiments. While it may be possible for single base pairs to part and swing out, this kind of opening would not show the salt effects observed. It seems more likely that the "breathing" of importance here involves a cooperative opening of several base pairs with some local moving apart of the backbone chains (see also Printz and von Hippel[1]).

Discussion.—Mechanism of exchange: Since the buried GC protons exchange, a

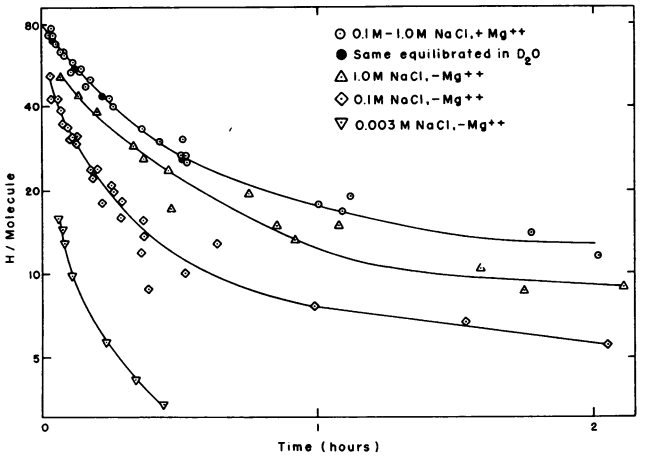

FIG. 2.—Hydrogen-tritium exchange of sRNA at pH 6.5, 4°, longer times.

certain amount of "breathing" must occur. Since addition of salts significantly slows *all* the hydrogens, the opening dependent mechanism must account for a significant fraction of the exchange. At pH 6.5, addition of Mg^{++} to sRNA in 0.1 *M* NaCl slows the exchange by a factor of 4. Even if, in the presence of Mg^{++}, all the exchange should proceed through Mechanism 1, then in 0.1 *M* NaCl without Mg^{++}, $^3/_4$ of the exchange would occur through Mechanism 2. Reduction of NaCl concentration makes the relative role of Mechanism 2 even greater.[18]

This argument can be taken further. At pH 6.5, the exchange curve found in the presence of Mg^{++} can be reproduced by multiplying the 0.1 *M* NaCl–no Mg^{++} curve, on the time axis, by a factor of 4, or the 1 *M* NaCl curve by a factor of 1.6. The shapes of these curves, therefore the distribution of rate constants k_c,k_1, and Kk_3 generating them, is identical. In view of the observed constancy of the ratio of rates, the sizable range of rates apparently present, and the fact that the absolute value of k_c (Mechanism 1) would be insensitive to salt concentration, it is unlikely that Mechanism 1 contributes significantly to the exchange seen under any of these conditions. Further considerations involving especially the pH dependence of exchange rates (not discussed here) suggest that all the exchange is of the Kk_3 type. If this is true, then the effect of added Mg^{++} is to multiply all values of K_t by a factor. Mg^{++} might achieve this result by raising the standard free energy of opening, ΔF°_t, by an increment δF°_t, as follows:

Without Mg^{++}:	$\Delta F^\circ_t = -RT \ln K_t$
With Mg^{++}:	$\Delta F^\circ_t + \delta F^\circ_t = -RT \ln K_t/4 = -RT \ln K_t + RT \ln 4$
Mg^{++} contribution:	$\delta F^\circ_t = RT \ln 4 = 750$ cal/mole.

Mole here refers to mole of "breathing units," and the value found is for the number average "breathing unit." If we assume that the sole action of Mg^{++} is to cancel the contribution of phosphate-phosphate repulsions to the breathing equilibrium, and take Warner's estimate[19] of the electrostatic free energy term per base pair for the poly A + U dissociation to be applicable here, we find the breathing unit to

consist of two base pairs under these conditions. Use of our and Warner's data at 1 *M* NaCl leads to just the same number. In view of the various assumptions and uncertainties involved, the exact values found are not very secure. For example, these values will be spuriously low if a moderate contribution to the exchange is made by a k_1 mechanism. They will be misleadingly high if we have entered the region where Mg^{++} not only neutralizes phosphate repulsions but also counteracts an increased opening tendency brought about by a partial titration (in the absence of Mg^{++}) at N_1 of A and C moieties. (DNA at pH 6.5 shows such an effect.[20]) Nevertheless, the result may be taken to indicate that the size of the breathing unit is small. Further, it may be hoped that the approach outlined[21] will allow the accurate estimation of the contribution of various factors to the thermodynamic stability of nucleic acids.

Helix and coil content: We find 77 hydrogen bonds per average 70 nucleotide molecule of mixed yeast sRNA. This number corresponds to 89 per cent of the ~87 hydrogens available for hydrogen bonding (2 for G, 1 each for A, U, and C; average nucleotide composition from compilation by Brown[22]). The 70-nucleotide model of Felsenfeld and Cantoni[4] consisting of three helical segments of differing stability would involve about 71 hydrogen bonds. The reasonable agreement indicates that we are not measuring C2′ ribose hydrogens.

Insofar as the commonly occurring bases are concerned, mixed yeast sRNA shows essentially perfect base-pairing composition.[22] The high degree of hydrogen bonding found indicates that no large quantity of pure, specific sRNA will deviate greatly in composition from proper base-pairing. This measurement also limits and defines the "looping out" that can be considered to exist in an sRNA molecule. About 8 common bases and ~6 odd bases (or their equivalent, in hydrogen bond content, of common bases) will fail to be involved in helix. If the terminal (C)CA bases are exempted, then from the body of largely double helical sRNA will protrude about 5 or 6 common bases and ~6 odd bases in non-hydrogen-bonded loop conformation(s). For an 85-nucleotide model, 2 more nucleotides per molecule would be available for loop formation.

These numbers are consistent with the suggestion of Cantoni *et al.*[6] that the odd bases function to mark off a looped-out single recognition site. On the other hand, these results can be taken to suggest, in addition, a similar construction for the activating enzyme recognition site. Each of the two loops might then consist of about three coding common bases with one or more odd bases on either side.

"Breathing" errors and structure: The "breathing" activity of nucleic acids, and proteins as well, may have fairly general implications for the functional activities of these molecules (e.g., see von Hippel and Felsenfeld[23]). With respect to the above, consideration of specific mechanisms by which these "breathing" reactions could produce errors in sRNA recognition functions suggests that sRNA recognition sites might well occur in loops of about the size and with the peculiar base distribution indicated. The "breathing" impresses a kind of indeterminacy limitation upon the accuracy with which a recognition site can be located. Thus if bases in recognition sites are flanked by *other common bases* in *conformationally equivalent situations*, the existence of overlapping, spurious sequences might produce occasional errors of "reading." Temporary "mistakes" in refolding during "breathing" might also produce reading errors. One possible way of averting this problem would be to

place each recognition sequence in a peculiar steric situation that identifies it unambiguously, as in a small loop, and to flank each sequence with a combination of odd bases which, taken together, neither participate in a spurious sequence nor allow errors in refolding.

It may be that the errors pictured here are biologically negligible and require no special structural adaptation. Then, presumably, the odd bases fulfill some other function. The odd bases have often been suggested to function either in stabilization of loop structures bearing recognition sites or as part of the recognition sequence itself. A great deal of experience with the renaturation of DNA,[24] with the spontaneous formation of structure by synthetic polynucleotides, not least with the rapid reformation of active structure by fully unfolded sRNA,[25] suggests that, especially in a polynucleotide as small as sRNA, *equilibrium* structure, both helix and loops, is predetermined by nucleotide sequence alone. Further, a number of experiments have shown unmethylated sRNA to be competent *in vitro*.[26-28] Thus, at least the methylated bases are not essential parts of coding sequences and are not important determinants of the integrity of putative recognition loops. It seems not unreasonable, then, that the odd bases function, at least in part, simply to avoid the relatively infrequent errors that might arise, in their absence, as a result of "breathing." Such low-level errors could pass unnoticed in *in vitro* experiments but might severely handicap a living organism.

The scheme discussed has the virtue of suggesting answers to certain current problems. The loops pictured place specific, to-be-methylated bases in an unambiguous and identifiable (by methylases) topological situation, at the junction between helix and coil. Methylating enzymes, which act on a limited number of bases in preformed sRNA[29] evidently respond to some such cue (for discussion see refs. 30 and 31). This suggests, by extension, that the puzzling patterns of methylation found for heterologous enzyme-nucleic acid systems might arise as a result of the *creation* of effective loops through adsorption of protein onto substrate nucleic acid (as elaborated by Felsenfeld *et al.*[32]), either enzyme protein itself or a fraction of the contaminant protein normally present.

The structure of a number of the odd bases, notably the 5-CH_3 derivatives and pseudouracil, is not obviously inconsistent with formation of double helix. If the scheme outlined is correct in detail, then they must perform the other function assigned, i.e., they must not be recognized as coding bases. This could only occur if either activating enzyme, or the messenger RNA-ribosome complex, or both, recognize the "back side" of bases (positions 5, 6, and 7 or 4) and reject the bases indicated.

It is of interest to note the consequences of these considerations for possible structural models of sRNA. sRNA is a single polynucleotide chain with unusual hydrodynamic asymmetry and rigidity.[7, 33] This, together with its high helical content[3, 4] (confirmed here), and X-ray diffraction results with crystalline sRNA fragments,[34] have led to the generally accepted picture of a chain bent back on itself to form a long length of double helix. The bend of this structure must include at least 3 non-hydrogen-bonded bases.[34] If, as suggested above, two recognition loops exist, then the estimate of the small number of bases available for loop formation suggests that one of the loops will be in the bend. That the messenger recognition site (anticodon) may be remote from the aminoacyl terminus is suggested by a number of experiments which have shown that, during protein synthesis,

messenger RNA is bound to the 30s ribosomal subunit,[35, 36] while sRNA and attached nascent protein are bound to the 50s subunit.[37] Thus the loop at the bend remote from CCA may well contain the anticodon.

It seems not unlikely that a loop in the middle of the long double helical body of sRNA would be inconsistent with its apparent rigidity. A loop could act as a permanently open nucleus from which waves of "breathing" might emanate to kink the helix. We cannot estimate the rate constant for the production of such kinks. However, it is clear that the repair of a kink would be a ponderous process involving reorientation of the total molecule. In any case, simple molecular stereochemical considerations suggest that, in the absence of compelling (and as yet unknown) reasons to the contrary, the activating enzyme recognition site ought not to be remote from the amino acid acceptor site, the CCA terminus. The experiment of Nihei and Cantoni[38] in which the appearance of bases freed by the *exonuclease* snake venom phosphodiesterase was followed, excludes odd bases from near the . . . CCA end, but, as indicated by Cantoni *et al.*,[6] this kind of observation becomes less restrictive farther away from the initiating end. Thus, in a once bent model we might speculate that the enzyme recognition loop will be found near the end of the . . . pGp limb and spatially close to the aminoacyl terminus (presumably about a half turn of helix removed from pGp to place it on the same side of the helix as CCA).

In a twice-bent model, the bends must be placed very asymmetrically. This restriction arises from the observation of Nihei and Cantoni that 40 per cent cleavage of sRNA by snake venom diesterase releases 90 per cent of the molecule's hypochromicity.[38] In this model, the above reasoning would suggest that the enzyme recognition site occurs in the bend nearer to the ends.

Summary.—Measurements of the hydrogen exchange behavior of mixed yeast sRNA are reported. Only hydrogens involved in hydrogen bonds exchange slowly enough to be measured. The mechanism of the exchange is discussed in terms of two possible pathways, one dependent on local opening or "breathing" of structure, and one involving direct exchange without "breathing." The response of the kinetic exchange curves to varying salt conditions rules out the latter pathway. The rates of exchange of various hydrogens, then, reflect the relative stabilities of various segments of the molecule. Observation of changes in rates may allow measurement of the contribution of various factors to the thermodynamic stability of nucleic acids. We find about 77 hydrogen bonds per average 70 nucleotide sRNA molecule indicating the involvement of about 82 per cent of the nucleotides in helix. (Compare the recent estimates of 76% helix and 71 H bonds/molecule by Felsenfeld and collaborators.[3, 4]) The non-hydrogen-bonded bases must "loop out" from the largely helical structure (except terminal (C)CA). The estimated number of "looped out" bases is consistent with the model of Cantoni *et al.*[6] but also with a model containing two small loops. Some possible implications of the "breathing" of sRNA for errors in recognition functions and for the design and function of loops and the odd bases are discussed.

We are grateful to Dr. R. W. Holley and Dr. G. L. Cantoni for generous gifts of purified sRNA.

* A preliminary account of this work was presented at the 48th Annual Meeting of the Federation of American Societies for Experimental Biology, April 1964, Chicago, Ill. This investigation

was supported by USPHS grant AM-07853 from the National Institute of Arthritis and Metabolic Diseases.

[1] Printz, M., and P. H. von Hippel, these PROCEEDINGS, **53**, 363 (1965).

[2] Englander, S. W., *Biochemistry*, **2**, 798 (1963).

[3] Felsenfeld, G., and G. Sandeen, *J. Mol. Biol.*, **5**, 587 (1962).

[4] Felsenfeld, G., and G. L. Cantoni, these PROCEEDINGS, **51**, 818 (1964).

[5] Greenman, D. L., F. T. Kenney, and W. D. Hicks, *Biochem. Biophys. Res. Commun.*, **17**, 449 (1964).

[6] Cantoni, G. L., H. Ishikura, H. H. Richards, and K. Tanaka, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 123.

[7] Luborsky, S. W., and G. L. Cantoni, *Biochim. Biophys. Acta*, **61**, 481 (1962).

[8] Ofengand, E. J., M. Dieckmann, and P. Berg, *J. Biol. Chem.*, **236**, 1741 (1961).

[9] Allen, E. H., E. Glassman, E. Cordes, and R. S. Schweet, *J. Biol. Chem.*, **235**, 1068 (1960).

[10] Holley, W., J. Apgar, B. P. Doctor, J. Farrow, M. A. Marini, and S. H. Merrill, *J. Biol. Chem.*, **236**, 200 (1961).

[11] Optical rotation and ultraviolet absorbance measurements at room temperature and below, with and without Mg^{++}, between pH 6.5 and pH 8.5 indicate no change in structure to occur over this range of conditions, so that the number of hydrogen bonds should remain constant at least over this range of conditions.

[12] It can be shown that, since free THO concentration in the sRNA band decreases exponentially with distance, therefore with time, in the column the corrected zero time occurs when the contaminating free tritium level has been reduced to 37% of its original value.

[13] This kind of mechanism has been considered for the hydrogen exchange of poly-D,L-alanine by Linderstrom-Lang,[14] of collagen by Bensusan and Nielsen,[15] and is discussed in considerable mathematical detail by Hvidt.[16] The equilibrium opening step of Mechanism 2 might be pictured as the low temperature analogue of the reversible folding-unfolding phenomenon that occurs in the region of a melting transition. Evidently, though, the size of the "breathing unit" is much smaller at low temperature.

[14] Linderstrom-Lang, K., in *Symposium on Protein Structure*, ed. A. Neuberger (London: Methuen, 1958), p. 23.

[15] Bensusan, H. B., and S. O. Nielsen, *Biochemistry*, **3**, 1367 (1964).

[16] Hvidt, A., *Compt. Rend. Trav. Lab. Carlsberg*, **34**, 299 (1964).

[17] Felsenfeld, G., and S. L. Huang, *Biochim. Biophys. Acta*, **51**, 19 (1962).

[18] Obviously, for parallel pathways, the faster path carries the larger traffic. The slow reaction is in no way rate-limiting.

[19] Warner, R. C., in *Informational Macromolecules*, ed. H. J. Vogel, V. Bryson, and J. O. Lampen (New York: Academic Press, 1963), p. 111.

[20] Printz, M., and P. H. von Hippel, in preparation.

[21] Hvidt has previously made use of the relationship between free energy and equilibrium constant of conformational transitions in a hydrogen exchange study of lysozyme.[16]

[22] Brown, G. L., in *Progress in Nucleic Acid Research*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1963), p. 286.

[23] von Hippel, P. H., and G. Felsenfeld, *Biochemistry*, **3**, 27 (1964).

[24] Marmur, J., and D. Lane, these PROCEEDINGS, **46**, 453 (1960).

[25] Takanami, M., T. Okamoto, and I. Watanabe, *J. Mol. Biol.*, **3**, 476 (1961).

[26] Starr, J. L., *Biochem. Biophys. Res. Commun.*, **10**, 181 (1963).

[27] Peterkofsky, A., C. Jesensky, A. Bank, and A. H. Mehler, *J. Biol. Chem.*, **239**, 2918 (1963).

[28] Littauer, U. Z., K. Muench, P. Berg, W. Gilbert, and P. F. Stahr, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 157.

[29] Fleissner, E., and E. Borek, these PROCEEDINGS, **48**, 1199 (1962).

[30] Borek, E., in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 139.

[31] Gold, M., and J. Hurwitz, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 149.

[32] Felsenfeld, G., G. Sandeen, and P. H. von Hippel, these PROCEEDINGS, **50**, 644 (1963).

[33] Otaka, E., and S. Osawa, *Nature*, **185**, 921 (1960).

[34] Spencer, M., W. Fuller, M. H. F. Wilkens, and G. L. Brown, *Nature*, **194**, 1015 (1962).
[35] Nirenberg, M. W., and J. H. Matthaei, these PROCEEDINGS, **47**, 1588 (1961).
[36] Takanami, M., and T. Okamoto, *J. Mol. Biol.*, **7**, 323 (1963).
[37] Gilbert, W., *J. Mol. Biol.*, **6**, 389 (1963).
[38] Nihei, T., and G. L. Cantoni, *J. Biol. Chem.*, **238**, 3991 (1963).

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*CONTROL OF VIRAL MESSENGER RNA AFTER LAMBDA PHAGE INFECTION AND INDUCTION**

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Communicated by R. H. Burris, December 18, 1964

Phage λ is an inducible temperate phage which provides an interesting model for study of the regulation of gene expression. When it infects a sensitive cell, the phage either enters the vegetative state where it multiplies actively and produces mature phage, or it enters the prophage state where it is incorporated into the bacterial genome and replicates with it.[1] In the vegetative state, the genetic information carried by the phage is expressed. In the prophage state, the only genetic information which is known to be expressed is that of the C_I region, which makes the lysogenic cell immune to homologous superinfecting phage. The prophage can be induced to enter the vegetative state and produce phage.

Jacob and Monod have interpreted the regulation of the λ genome in terms of their general model for regulation of protein synthesis.[2] In this model, gene expression is controlled primarily at the level of transcription of DNA into messenger RNA. They suggested that the C_I region of λ directs the synthesis of a repressor which, by virtue of its affinity for certain sites on the λ chromosome, blocks the transcription of regions of the chromosome into messenger RNA. In these terms, the λ repressor controls the expression of most of the λ genes, and induction of the prophage to undergo vegetative development is the result of inactivation or removal of the repressor. Immunity of the lysogenic cell to superinfection is due to repression of the superinfecting λ chromosome. Since λ messenger RNA synthesis is the proposed point of regulation by repressor, expression of the λ genes should lead to increased levels of λ specific messenger RNA. The experiment of Attardi *et al.*[3] showed that following induction of a lysogenic culture, the λ messenger RNA levels were substantially elevated, compared to the uninduced levels. In this experiment, it is likely that there was an increase in the number of λ copies per cell, and it is therefore possible that the changes seen are due to an increasing gene dosage.

The present series of experiments are designed to study λ messenger RNA levels in various phases of the life cycle of λ. The chief questions asked are: (1) Do prophage genes function to produce messenger RNA in the uninduced state? (2) How does the level of messenger RNA change when the prophage is induced to undergo vegetative development? (3) How do messenger RNA levels change as the result of infection of a sensitive cell? (4) How are the changes seen in infection modified by immunity of the lysogenic host to the superinfecting phage?

In these experiments, the criterion for λ-specific messenger RNA was H^3-RNA