10. J. A. M. Hinke, in *Glass Microelectrodes*, M. Lavallee, O. F. Schanne, N. C. Hebert, Eds. (Wiley, New York, 1969).
11. W. McD. Armstrong and C. O. Lee, *Science* **171**, 413 (1971).
12. B. P. Nicolsky, *Zh. Fiz. Khim.* **10**, 495 (1937).
13. G. Eisenman, D. O. Rudin, J. U. Casby, *Science* **126**, 831 (1957); A. A. Lev, *Nature* **201**, 1132 (1964).
14. J. F. Quay and W. McD. Armstrong. *Proc. Soc. Exp. Biol. Med.* **131**, 46 (1969); *Amer. J. Physiol.* **217**, 694 (1969); J. F. White and W. McD. Armstrong, *ibid.* **221**, 194 (1971).
15. R. A. Robinson and R. H. Stokes, *Electrolyte Solutions* (Butterworths, London, 1965).
16. Although Na^+ influx from lumen to cell is increased in the presence of 3-*O*-methyl glucose (*2*), an increase in a_{Na} under these conditions seems unlikely because of the concomitant increase in cell water (*5*, *6*). The observed decrease in a_{Na} (Table 1) is easily interpreted in terms of the energy requirement (w) for net Na^+ transport from the cell interior to the serosal medium. For the reversible transfer of 1 gram equivalent of Na^+ under steady state open circuit conditions

$$w = RT \ln (a_{Na}^{o}/a_{Na}) + E_s F \quad (3)$$

where a_{Na}^{o} is the extracellular Na^+ activity, E_s is the serosal membrane potential, and R, T, and F have their usual meanings. If it is assumed that the supply of metabolic energy and its coupling to Na^+ "pumping" are unaffected by the actively transported organic solute, the known decrease in E_s under these conditions (*14*) would permit the maintenance of a lower steady state a_{Na} since the other parameters of Eq. 3 are unchanged. The increased short circuit current (under conditions where this reflects net transcellular Na^+ transport) induced by actively transported solutes (*2*) is also consistent with a decrease in a_{Na}. "Short circuiting" means abolishing $(E_s - E_m)$, the transmural PD. Hence, in this condition, E_m can be substituted for E_s in the electrical work term of Eq. 3. Since E_m is significantly reduced by actively transported solutes (*14*), the electrical work required for net Na^+ transport from the cell interior to the serosal medium will be correspondingly deduced. Thus, for a given energy input one can expect an increased transcellular movement of Na^+.

17. Although these estimates are consistent with those reported for the same parameters in other cells (*7*), they must be regarded as highly tentative and subject to further experimental verification. Both the assumptions implicit in Eqs. 1 and 2, that is, $g_c = g_m$ and $\beta_K \sim 0$, are open to question. In particular, the second assumption which maximizes the value of α obtained from Eq. 1, may not be true for all experimental conditions (*11*).
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Messenger RNA Structure: Compatibility of Hairpin Loops with Protein Sequence

Abstract. *Examination of the amino acid sequences of human cytochrome c and the α-chain variant of human hemoglobin Constant Spring has revealed the possibility for base-paired hairpin loops in the messenger RNA's for these proteins. A similar analysis of the bacteriophage R17 coat protein suggests an additional unobserved loop in the R17 RNA. If such loops are present in messenger RNA's generally, it would suggest that DNA has more than one stable base-paired conformation.*

A number of RNA's appear to possess secondary structure in the form of base-paired hairpin loops. Transfer RNA's (tRNA) are the most familiar examples (*1*), but 5*S* ribosomal RNA of *Escherichia coli* has these properties (*2*) as does an RNA of unknown function from human cells infected with adenovirus 2 (*3*). Most surprising, however, has been the demonstration of several loops in the segment of RNA that codes for the coat protein of R17 bacteriophage (*4*). These hairpin loops imply that the base sequence of the messenger RNA (mRNA) is dictated by its ability to form stable base-paired loops, as well as by the functional tertiary structure of the coat protein. At first glance this combination of restriction on mRNA structure might seem incompatible; however, the redundancy in the genetic code permits extensive alteration of base sequence to achieve base-pairing without greatly altering the amino acid sequence. If occasional unpaired bases or shifts in the register such as those that occur in R17 RNA (*4*) are permitted, the potential for loop formation is further increased.

We have, with the aid of a computer program, examined several proteins with the object of discovering regions compatible with base-paired loops in the corresponding mRNA. The process involves translating the protein amino acid sequence into an RNA sequence that includes all the redundancies in the genetic code. This RNA sequence is compared with itself in reverse polarity in all possible linear registers. Base comparisons are recorded as a match if they are complementary, a potential match if one of the redundancies permits base-pairing, and no match if there is no possibility for complementary pairing. Potential loops on the computer printout are recognized by long runs of matches or potential matches, with few gaps or register shifts. The program thus eliminates all regions where loops cannot occur but does not uniquely predict the existence of loops, only the possibility for loops.

Our analysis of the R17 coat protein has revealed all the internal loops in the mRNA observed experimentally by Sanger (*4*). In addition, several loops suggested by the program were shown to be spurious upon examination of the known base sequence. One of the interesting results of analysis of the R17 coat protein is the indication of a loop in the mRNA coding for residues 12 to 23, a region of R17 RNA that has not yet been sequenced. This region is presented in Fig. 1a along with the RNA base sequence predicted, provided that there is a base-paired loop in this region. Twelve previously undefined bases in this sequence are predicted by this analysis, leaving only one unspecified base at an unpaired position at the closed end of the loop.

The spatial restrictions for packing RNA in the R17 bacteriophage capsule might represent a strong selective pressure for the formation of base-paired loops. Such restrictions would not exist for mRNA's in bacteria and higher organisms. The loops may have a more general function such as the stabilization of RNA; therefore, we have examined human cytochrome c (*1*) and the recently described chain termination variant (Constant Spring) of the α chain of human hemoglobin (*5*). There are several amino acid sequences in these proteins which are compatible with base-paired loops in their mRNA. Selected regions are illustrated in Fig. 1b for cytochrome c and Fig. 1c for the hemoglobin α chain.

The regions chosen have particular theoretical importance. Cytochromes c from a diversity of organisms possess a sequence of 11 invariant amino acids (*1*). The evolution of a loop in the RNA coding for this region might tend to stabilize the amino acid sequence. Two possible loops for this region are presented in Fig. 1b. The first example is in a "1-1" register, that is, the first base of triplet codons on opposite sides of the loop are aligned. The second example is in a "2-2" register. In general, loops with "3-3" registers are considered less likely since they do not take full advantage of the redundancy in the third position of most codons.

The discovery of a chain termination variant for the α chain of human hemoglobin (*5*) has enabled us to examine a portion of the hemoglobin messenger not normally translated into protein. The additional 31 amino acids found in this variant represent a segment of mRNA whose base sequence has been free to evolve toward the formation of

very stable base-paired loops (if such is evolutionarily desirable), since the back selection pressure resulting from the restrictions of stable protein conformation is lacking. This region, more than any other in the hemoglobin molecule, shows strong potential for base-paired loops. Most intriguing are the two presented in Fig. 1c. In one case the normal hemoglobin chain termination codon falls at the top of the loop, whereas the other potential loop begins with the chain termination codon. The latter loop is notable for the abundance of guanine • cytosine (G • C) pairs (19) relative to the weaker adenine • uracil (A • U) (7) and G • U (2) pairs (*6*). The 1-1 register of this loop shifts to a 2-2 register by the exclusion of the second base of the codon for alanine 155.

These examples demonstrate rather clearly a potential advantage of the triplet code with numerous redundancies (*7*). A doublet code is capable of coding for 16 different amino acids, but because of a lack of redundancy it would be difficult to form base-paired loops in mRNA and yet maintain the base sequence needed to specify a functional protein. The redundancies in the triplet code, however, permit mRNA sequences that satisfy requirements for both protein structure and RNA structure. If hairpin loops do indeed exist in mRNA, recent assertions that mutations in redundant bases (synonymous mutations) are selectively neutral (*8*) may have to be modified.

The hypothesis that mRNA's in general have base-paired loops carries the rather thought-provoking implication that the DNA's from which they are transcribed may also possess this potential property in both strands. Normally such loops in DNA would be less stable than the linear, fully paired double helix unless an unwinding force

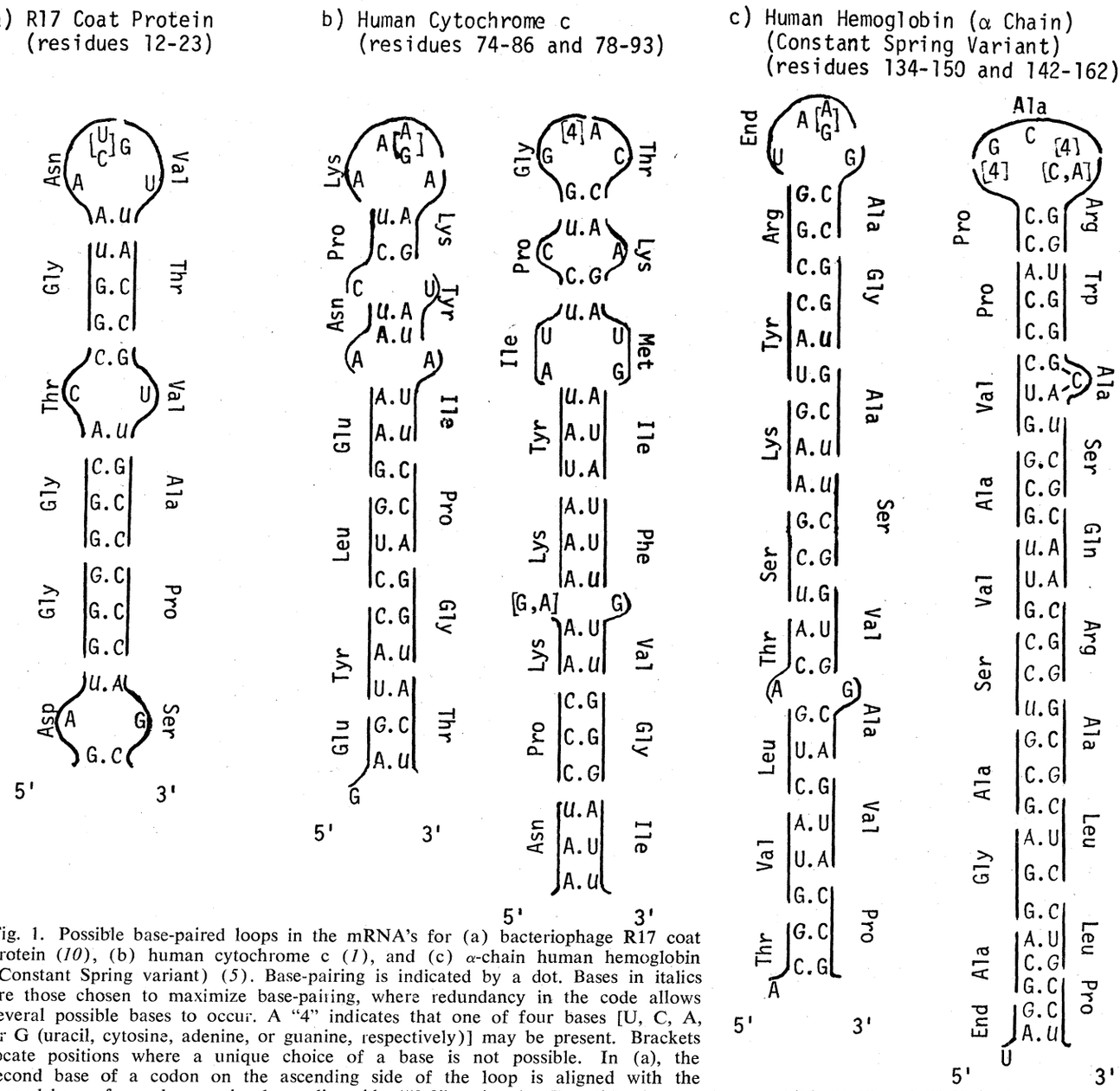

Fig. 1. Possible base-paired loops in the mRNA's for (a) bacteriophage R17 coat protein (*10*), (b) human cytochrome c (*1*), and (c) α-chain human hemoglobin (Constant Spring variant) (*5*). Base-pairing is indicated by a dot. Bases in italics are those chosen to maximize base-pairing, where redundancy in the code allows several possible bases to occur. A "4" indicates that one of four bases [U, C, A, or G (uracil, cytosine, adenine, or guanine, respectively)] may be present. Brackets locate positions where a unique choice of a base is not possible. In (a), the second base of a codon on the ascending side of the loop is aligned with the second base of a codon on the descending side ("2-2" registry). For (b), the two loops exhibit "1-1" and "2-2" registry, respectively. The long loop in (c) shifts from "2-2" to "1-1" registry by the exclusion of a cytosine on the descending side of the loop. Residue numbers are taken from (*1*). "End" is the chain termination codon which has mutated in the Constant Spring variant.

were applied to the DNA strand. In such a case the strain of an untwisted helix could be relieved or accommodated by the formation of hairpin loops. This property might be important in the structure of chromosomes. The existence of such a loop at the *E. coli* lactose operator gene might be a simple explanation for the completely symmetrical genetic map determined for physiologically similar operator constitutive (O^c) mutants (*9*).

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References and Notes

1. M. O. Dayhoff, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Md., 1969), vol. 4.
2. G. G. Brownlee, F. Sanger, B. G. Barrell, *J. Mol. Biol.* **34**, 379 (1968).
3. K. Ohe and S. M. Weissman, *J. Biol. Chem.* **246**, 6991 (1971).
4. F. Sanger, *Biochem. J.* **124**, 833 (1971).
5. J. B. Clegg, D. J. Weatherall, P. F. Milner, *Nature* **234**, 337 (1971).
6. C. Delisi and D. M. Crothers, *Proc. Nat. Acad. Sci. U.S.* **68**, 2682 (1971).
7. Other aspects of the triplet code are elaborated → F. H. C. Crick [*J. Mol. Biol.* **38**, 367 (1968)] ; → L. E. Orgel (*ibid.*, p. 381).
8. M. Kimura, *Genet. Res.* **11**, 247 (1968); J. L. King and T. H. Jukes, *Science* **164**, 788 (1969).
9. J. R. Sadler and T. F. Smith, *J. Mol. Biol.* **62**, 139 (1971); A. Gierer, *Nature* **212**, 1480 (1966).
10. K. Weber, *Biochemistry* **6**, 3144 (1967).
11. Supported by University of Delaware.

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Iodine Metabolism: Preferential Renal Excretion of Iodide Derived from Triiodothyronine Deiodination

Abstract. *Measurements were made in rats of the relative rates of accumulation in urine or in the thyroid of radioactive iodide derived from simultaneous injections of ^{131}I-labeled triiodothyronine and ^{125}I-labeled iodide. The data indicate that deiodination of triiodothyronine by the kidney results in a loss into the urine of iodine which does not enter the general body iodide pool. This renal "iodide leak" should be considered in kinetic models of iodine metabolism.*

Several mathematical treatments of the kinetics of iodine metabolism have been carried out in recent years. The major pathways have been considered to be relatively uncomplicated (*1*). In all these schemes it has been assumed that the iodide generated through deiodination of thyroid hormones or other iodoamino acids in extrathyroidal tissues has the same metabolic fate as that of iodide already present in the plasma or tissue.

The kidney is a particularly active organ in the deiodination of thyroid hormones (*2*). We considered it possible that a portion of the iodide generated there might enter the renal tubule lumen and not be readily absorbed. Instead of entering the plasma iodide pool by way of the renal venous effluent, some iodide generated de novo in the kidney might thus be excreted immediately and not enter the general body iodide pool. An analogy can be found in the effect of the parathyroid hormone on the adenyl cyclase system of the kidney. Parathormone causes a marked increase in the amount of adenosine 3′,5′-monophosphate (cyclic AMP) excreted by the kidney without inducing any significant rise in the concentration of cyclic AMP in the plasma or any change in the renal clearance of this nucleotide (*3*).

In an effort to evaluate the validity of our hypothesis, we simultaneously injected intraperitoneally tracer doses of carrier-free ^{125}I-labeled iodide and doses of ^{131}I-labeled triiodothyronine (T_3) (0.1 to 1 μg) (*4*) into male Sprague-Dawley rats weighing approximately 200 g each. Each animal thus served as its own control. The quantity of radioactivity was equal (1 to 50 μc) for both isotopes in each rat. Triiodothyronine was chosen rather than thyroxine (T_4) since metabolism of T_3 is much more rapid than that of T_4 so that a major fraction of T_3 would be expected to undergo deiodination in a few hours. We carried out five separate experiments with three to six rats in each experiment. We caused some animals to be made iodine-deficient by feeding them 0.15 percent (by weight) propylthiouracil in a low-iodine diet (LID) (*5*) for 1 week and then feeding them LID without propylthiouracil for 7 to 10 days before the studies were made. Other animals were fed a high-iodine diet of Purina Lab Chow (*6*).

After injection of the radioisotopes, the animals were placed in individual metabolism cages without food or water and urine samples were collected every 2 hours for 6 hours. The animals were then killed. The thyroids were removed, their content of both isotopes was determined, and they were digested and chromatographically analyzed in two different solvent systems as described earlier (*7*). The radioactivity in the urine was similarly analyzed, but in this case direct chromatography was carried out without digestion.

The results of all three LID experiments were similar, as were those of the Purina experiments. The data are pooled in Table 1. More than 95 percent of both ^{131}I and ^{125}I radioactivity in the urine was iodide except in one rat in which 70 percent of the urine ^{131}I radioactivity corresponded to T_3. The data from this animal have been excluded from Table 1. The urine volume remained constant at approximately 1.5 ml per rat for each 2-hour collection period throughout the experiment. The distribution of radioactivity in the chromatographs of the thyroid digests in each experiment was similar for both isotopes and indicated that there was no $[^{131}I]T_3$ in the thyroid beyond that which was due to the iodination of thyroglobulin with ^{131}I-labeled iodide.

The urine/thyroid (U/T) radioactivity ratios were used as the index of comparison of the two isotopes. Equality of the ratios would indicate that the iodide generated from T_3 deiodination was metabolized identically with that originally injected as iodide. Inequality would indicate that one iso-

Table 1. Percentage of radioactivity injected as $[^{131}I]T_3$ or as ^{125}I-labeled iodide accumulated in urine or thyroid in 6 hours. Results are given as the pooled mean ± the standard error of the mean of three LID and two Purina experiments. There was a pooled total of nine rats in each of the groups.

Diet	Label	Urine (% of dose)	Thyroid (% of dose)	^{131}I U/T / ^{125}I U/T
LID	^{131}I	2.7 ± 0.4	22.1 ± 3.2	1.90 ± 0.21
LID	^{125}I	5.9 ± 0.6	83.8 ± 3.2	
Purina	^{131}I	5.6 ± 0.7	1.3 ± 0.1	1.39 ± 0.08
Purina	^{125}I	28.7 ± 2.1	9.5 ± 0.5	