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22. O. Nolting and D. Arnold, Eds., *Municipal Year Book 1966* (International City Manager Association, Chicago, 1966), p. 90. Table 2 shows the distribution of form of government by city size.
23. L. Gulick, *Public Admin. Rev.* **17**, 106 (1957).
24. For a discussion of alienation as a political concept, see W. Thompson and J. Horton, *Social Forces* **38**, 190 (1959–1960); J. Horton and W. Thompson, *Amer. J. Sociol.* **67**, 485 (1962); C. Stone, *Public Opin. Quart.* **29**, 213 (1965).
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28. B. Mausner and J. Mausner, *Sci. Amer.* **192**, 35 (1955).
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33. For survey reports and summaries, see National Association of Science Writers, *Science, the News, and the Public* (New York Univ. Press, New York, 1958); and W. Schramm, *Science and the Public Mind* (AAAS, Washington, D.C., 1962).
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35. L. Harris, cited in U.S. Public Health Service, *Emphasis: Fluoridation* (U.S. Government Printing Office, Washington, D.C., 1966), p. 5.
36. A. S. Metz, *Social Forces* **44**, 477 (1966).
37. J. Mueller, *West. Polit. Quart.* **19**, 59 (1966).
38. There have been a number of fluoridation campaign studies. Particularly useful is I. Sanders, *J. Social Issues* **17**, 55 (1961); W. Gamson, *Trans-Action* **2**, 9 (1964).
39. H. Raulet, *J. Social Issues* **17**, 41 (1961).
40. W. Gamson, *Health Educ. J.* **19**, 51 (1961), Table 3.
41. The figures are 74 percent favorable with incorrect information and 70 percent favorable with correct information (*36*).
42. For example, R. Bauer, I. de S. Pool, L. Dexter, *American Business and Public Policy* (Atherton Press, New York, 1963).
43. J. Steiner and F. Stare, in U.S. Public Health Service, *Emphasis: Fluoridation* (U.S. Government Printing Office, Washington, D.C., 1966).
44. Among the leading antifluoridationists are G. L. Waldbott, B. Exner, C. Brusch, J. Forman, C. Fredericks, L. Gross, V. O. Kurme, R. Lee, L. Spera; see reports on each of these individuals in Bureau of Public Information, *J. Amer. Dent. Ass.* **71**, 1155 (1965).
45. As Mueller (*37*) notes, these data do not support Gamson's (*29*) suggestion that these persons oppose fluoridation because the measure somehow symbolizes the buffeting about one takes in a society where not even the water one drinks is sacrosanct.
46. L. Terry, in U.S. Public Health Service, *Emphasis: Fluoridation* (U.S. Government Printing Office, Washington, D.C., 1966), p. 4.
47. For a history of this program see R. Walder and R. Williams, *Enrichment of Flour* (National Research Council, Washington, D.C., 1944).
48. Recommendations of the First National Conference on Fluoridation, in *Emphasis: Fluoridation*, (U.S. Government Printing Office, Washington, D.C., 1966), p. 17.
49. J. Schumpeter, *Capitalism, Socialism, and Democracy* (Harper, New York, 1950), chaps. 21 and 22.
50. Even legislatures may be unable to deal with difficult scientific issues. See S. Reiser, in *Knowledge and Power*, S. Lakoff, Ed. (Free Press, New York, 1966), pp. 293–311.

NEWS AND COMMENT

1968 Nobel Laureate in Medicine or Physiology

The Nobel Prize in Medicine or Physiology for 1968 has been awarded, jointly, to Robert W. Holley of the Salk Institute, Har Gobind Khorana of the University of Wisconsin, and Marshall W. Nirenberg of the National Institutes of Health.

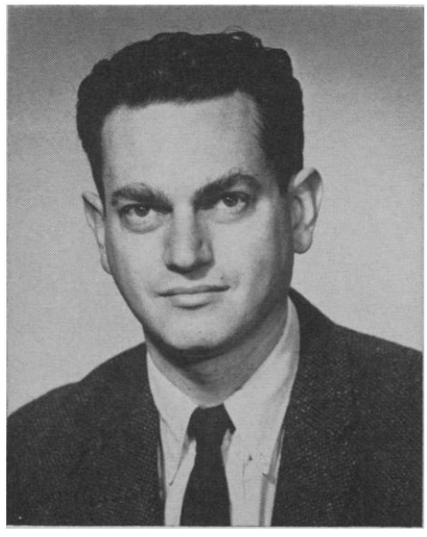
The announcement of the awards emphasized that each man was recognized for work carried out independently of the other two. But the work of the three is interrelated, and the significance of each achievement is enhanced by the achievement of the others. These three men together constitute a triplet of great sense.

It is difficult to decide where to start the story of the genetic code—no point in time is really a beginning. The acceleration of research in this area does, however, seem to coincide with the initial experiments reported by Nirenberg and his co-worker Heinrich Matthaei (now of the Max-Planck Institute, Göttingen). Nirenberg was interested in the chemical mechanism underlying the well-documented theory that what is specified by the genes is the structure of proteins. How, within a cell, does the linear sequence of the four nucleotides in the DNA structure specify the linear sequence of a protein? A protein contains a linear arrangement of amino acids held together by covalent bonds. The structural uniqueness of a protein, and consequently the uniqueness of its function, is defined by the number and linear order of the 20 possible amino acids. Nirenberg and Matthaei made a crude, cell-free preparation from the bacterium *Escherichia coli* and looked for protein synthesis that was dependent on the addition of nucleic acid. Given the complexity and crudity of the system, the concept seemed then, and even now, too simple. But it worked. The addition of nucleic acid did indeed stimulate the incorporation of amino acids into protein.

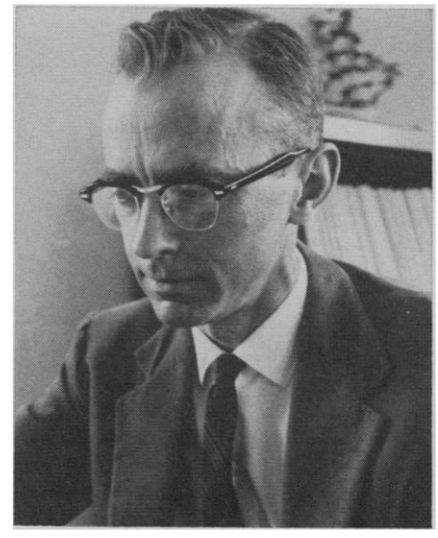
The active nucleic acid was not DNA, but the chemically related nucleic acid, RNA. [That RNA served to relay the genetic information was consistent with the theory proposed by Jacob and Monod in 1961. These men, Nobel laureates themselves (1965), proposed that an RNA, messenger RNA, containing a replica of the sequence of the DNA, actually functions in defining amino acid sequences.] Most surprisingly, however, natural RNA containing the four common ribonucleotides [adenylic (A), uridylic (U), cytidylic (C), and guanylic (G) acids] was not necessary in Nirenberg's cell-free system. An RNA-like polyribonucleotide containing only one of the ribonucleotides, uridylic acid, was extremely active. Furthermore, polyuridylic acid stimulated the incorporation of only one of the 20 possible amino acids, namely phenylalanine. The "protein" being made was composed exclusively of phenylalanine units. Nirenberg and Matthaei concluded that one or more uridylic acid residues represented the code for phenylalanine.

When, in the spring of 1961, these results became known to some of Nirenberg's colleagues at the NIH, the excitement ran high. And the formal announcement of the results at the International Biochemistry Congress in Moscow that summer was electrifying. The possibility of determining the entire code was clear.

The extension of the experiment to other amino acids required a variety of polyribonucleotides containing the other common ribonucleotides, either singly or in combinations. Such polymers, while not widely available, could be prepared with polynucleotide phosphorylase. This enzyme had been discovered in 1955 by Grunberg-Manago and Ochoa, and it was for this finding, in part, that Ochoa shared a Nobel Prize in 1959. The enzyme catalyzes the synthesis of polyribonucleotides of any de-



Marshall W. Nirenberg



Robert W. Holley

sired composition: if more than one type of ribonucleotide is used, the order of the different nucleotides in the resulting polymer is random. Ochoa had early obtained the collaboration of Leon Heppel of the NIH, to study and characterize the polymers made by the enzyme. At that time, Heppel was one of the few men in the world who knew much about handling polyribonucleotides. Therefore, by 1961, the materials and know-how that Nirenberg required were at hand. Heppel, as well as others at the NIH, became enthusiastically involved in aiding Nirenberg's effort. Ochoa, too, was in a good position to extend Nirenberg's observations. And by early 1963, similar results in both Nirenberg's and Ochoa's laboratories had defined the nucleotide composition of the triplet codewords for the 20 amino acids. The triplet code had been strongly suggested by genetic experiments published in 1961 by Francis Crick (Nobel Laureate, 1962) and his colleagues.

Soon after the discovery that polyuridylic acid specified phenylalanine, Nirenberg's laboratory reported that another type of cellular RNA, called transfer RNA or tRNA, was an obligatory intermediate in the formation of polyphenylalanine. In any cell there are many types of tNRA molecules, and each type is specific for a particular amino acid.

The initial indication of the existence of these tRNA's, and their possible involvement in protein synthesis, was reported by Robert Holley early in 1957. By 1961, it was known that a series of enzymes, each specific for one amino acid and one particular tRNA, catalyzed the formation of a covalent linkage between the amino acid and the tRNA's. It was widely believed, even before Nirenberg's experiments, that these compounds donated their amino acids directly during protein synthesis.

Early work on the mixture of all tRNA's indicated that all the molecules were (i) relatively short—not more than 100 nucleotides per chain; (ii) similar, if not identical, in the nucleotide composition at the two ends of the chains; (iii) unusual in that they contained a surprising number, albeit very small amounts, of nucleotides other than the four common ones.

Most people stopped at this point. To go further and attempt to elucidate the actual sequence of the nucleotides in any particular tRNA was a job too formidable for most. The problems were manifold, and each required major technical innovations. The first problem was how to purify one type of tRNA molecule free of all the others. It was plain that the differences between the molecules would be subtle, and pertinent methodology was almost nonexistent. Given a pure preparation of one tRNA, it was not even clear that the base sequence could be defined with the available methods.

With this background in mind, one can appreciate the reaction of the interested community to Holley's announcement early in 1965 that, after 5 years of work, he and his colleagues knew the primary structure—that is, the complete nucleotide sequence—of a tRNA specific for the amino acid alanine. The pure tRNA was obtained by the countercurrent-distribution technique. The procedures used to determine the structure were similar in concept to those introduced by F. Sanger (also a Nobel Laureate) for the determination of the sequence of amino acids in proteins. Two enzymes were used to break the tRNA chain into small pieces. These particular enzymes catalyze chain scission only at bonds occupied by a particular type of nucleotide residue: one enzyme, ribonuclease T_1 for example, splits at every guanylic acid residue; the other, pancreatic ribonuclease, splits at both uridylic and cytidylic acid residues. Enzymatic digestion thus generated a unique family of small polynucleotides for each of the specific enzymes used. Holley and his colleagues then worked out methods for separating each family into its constituent polynucleotides. The sequences of the pure, small polynucleotides were then determined. Some methods were available for this purpose, but many new ones had to be devised.

Now the pieces had to be put in a unique linear array. Because the two enzymes broke the original chain at different bonds, the small polynucleotides obtained from the two different digestions should have contained overlapping sequences. Had the chain contained only the four common nucleotides, unique overlaps might have been impossible. But the presence of a limited number of unusual nucleotides in the alanine tRNA was helpful. Some long pieces were put in unique order.

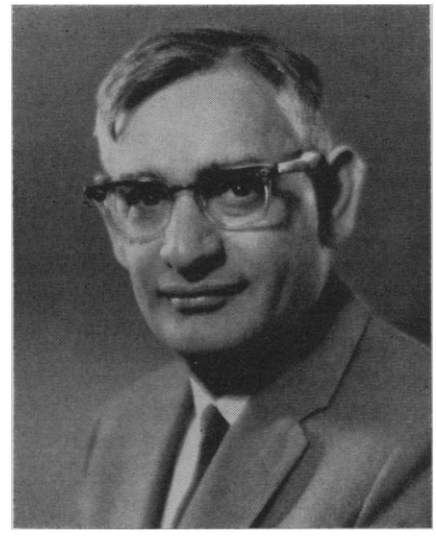
Holley and his colleagues then recognized that, under conditions known to maintain the secondary structure of the tRNA molecules, many of the normally susceptible bonds were not split by ribonuclease T_1. The term secondary structure refers to the configuration assumed by the linear molecule and may involve folding of the chain as well as the existence of helical regions. With alanine tRNA, conditions were found such that ribonuclease T_1 split only one bond in the entire chain. Other conditions gave only two splits, and so on. The smaller polynucleotide sequences could then be assigned to particular regions of the molecule, and ultimately the whole linear sequence could be written out.

Linear sequences are, however, only a beginning. The secondary structure is also crucial in understanding function.

Holley and his colleagues took the results of the limited digestion of alanine tRNA by ribonuclease T_1 as clues to secondary structure. They argued that, under conditions that maintain secondary structure, those bonds susceptible to digestion must be in exposed positions. Unexposed areas were assumed to be involved in double-helical configurations of the Watson-Crick type. Using these considerations Holley proposed several plausible models for the configuration of alanine tRNA. One of these, the cloverleaf model, has gained wide acceptance. This acceptance arises from the fact that all of the tRNA sequences now known (and about half a dozen have been elucidated since 1965) fit readily into a similar cloverleaf form. A second compelling argument for the cloverleaf structure is that in each case a suitable anticodon exists in an exposed portion of the molecule.

The new term, the anticodon, will have to be explained. Even before the elements of the code were known, Crick had proposed that some adapter must act as a translator between the code inherent in nucleic acid structure and the amino acids themselves. Specific interactions between nucleic acid components, by paired nucleotide, hydrogen-bonding (of the Watson-Crick type) were well known. On the other hand, specific stereochemical interactions between amino acids and nucleotides, in which amino acids "recognize" a polynucleotide structure, were unknown and unlikely. With the proof that amino acids in aminoacyl-tRNA's were the immediate precursors of protein amino acid, it was easy to view the tRNA molecules as the proposed adapters. These adapters are believed to function in the following way. A trinucleotide sequence in the tRNA, called the anticodon, is complementary (in the Watson-Crick sense) to the trinucleotide codon in the messenger RNA. The recognition of the codon message is attributed to the anticodon. The tRNA, since it carries the amino acid specific for that codon-anticodon pair, ensures insertion of the proper amino acid. An exposed position of the anticodon in the tRNA structure is then of obvious importance.

From the work with polyribonucleotides of random sequence, only the nucleotide composition, but not the order of the nucleotides, in the various triplet codons was known. For example, adenylic, guanylic, and cytidylic acids were



Har Gobind Khorana

in the codon for aspartic acid; but whether the codon was AGC, or CGA, or GAC, or other, could not be determined. In 1964, Nirenberg with Philip Leder, one of the large number of outstanding young men who had joined him during these years, made an unexpected discovery which allowed, with surprising ease, the determination of the sequence of nucleotides within the triplet. The initial finding, made simultaneously in several laboratories, was that a particular aminoacyl-tRNA would bind to ribosomes in the presence of a polyribonucleotide containing the corresponding triplet. For example, phenylalanine-tRNA binds to ribosomes only in the presence of polyuridylic acid. These observations were made by tedious and time-consuming procedures. Leder and Nirenberg discovered that, while aminoacyl-tRNA itself does not stick to disks of nitrocellulose, it does stick when bound to ribosomes, which themselves adhere to the disks. They quickly learned that even a trinucleotide—the isolated triplet itself—would permit the binding of the aminoacyl-tRNA. In a simple example, triuridylic acid (UUU) permits binding of phenylalanine-tRNA, while triadenylic acid (AAA) permits binding of lysine-tRNA. It was evident that, by testing a large number of the 64 possible trinucleotides, the actual codewords could be determined. The trinucleotides were readily prepared by enzymatic procedures; with rather astonishing speed, the majority of the triplets were determined.

It might appear from the foregoing that the story has been told, and without mention of the work from Gobind Khorana's laboratory. With a little thought, however, it will be apparent that several important pieces are missing. Prior to 1960, only the outlines of nucleic acid structure were understood, and the limited technology for studying the structure was familiar to few. The synthetic approach to nucleic acid structure was developed by Khorana. The chemical synthesis of polynucleotides—both the ribose and deoxyribose series—is an almost exclusive achievement of his laboratory. When Nirenberg and Leder discovered the nitrocellulose binding technique, Khorana and his colleagues were able to extend the binding experiments by testing each of the 64 possible ribonucleotides; all of the triplets had been chemically synthesized. Much earlier, Khorana had realized what might be accomplished if, instead of a random distribution of nucleotides, long-chain polyribonucleotides with defined sequences could be tested in the cell-free system of Nirenberg and Matthaei. The synthesis of such molecules in the ribose series (RNA-like polymers) is extremely difficult because of the complexities introduced by the 2′-hydroxyl group of the ribose (the normal internucleotide bond being 3′ to 5′). In the deoxyribose series (DNA-like polymers) the 2′-carbon of the ribose has no hydroxyl group, and chemical syntheses are thereby enormously simplified (but not simple).

In an elegant combination of synthetic and enzymatic methods, the problems in the chemical synthesis of polyribonucleotides were bypassed. Synthetic polydeoxyribonucleotides of known sequence were used to direct the synthesis of long, complementary polyribonucleotides, in reactions catalyzed by the enzyme RNA-polymerase. For example, RNA-like polymers with alternating sequences, such as -UCUCUC-UC-, were prepared. Such a polymer contains the alternating triplets UCU and CUC. Khorana and his colleagues demonstrated that this polymer directs the synthesis of a polypeptide with alternating amino acids—leucine and serine. A large number of such polymers were tested. The results were extended with polymers containing repeating tetranucleotide sequences and thereby a repeating array of four triplets; the resulting polypeptides contained a repeated tetraamino acid se-

quence. This work afforded unequivocal proof of codon assignments.

It also permitted the definition of some codons that had not previously been determined. Other of the triplets, which had seemed to code for no amino acid, were shown to serve as punctuation for the initiation and termination of one polypeptide chain. These latter assignments had already been suggested by genetic and biochemical experiments. The experiments from Khorana's laboratory also supplied proof of other attributes of the code. They demonstrated directly, in a way that the genetic experiments could not, that three nucleotides specify an amino acid. They proved the direction in which the information of the messenger RNA is read, that punctuation between codons is unnecessary, and that the codewords cannot overlap. Furthermore, because of the way the polyribonucleotides were prepared, these experiments showed that the sequence of nucleotides in DNA does indeed specify the sequence of amino acids in a protein, and it does so through the intermediary of an RNA.

These then are the extraordinary accomplishments of three men. Other investigators made findings essential to the total picture, and, together with these three, many have contributed to aspects of the story which are not recounted here.

The story of the genetic code unfolded in a reasonable and logical manner. At many points, the plausible experimental approach was apparent to many. The achievements of Holley, Khorana, and Nirenberg show common attributes that set these men apart from the many. Their separate triumphs are a combination of elegant scientific insight and style with the courageous daring and determination of the frontiersman.

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George C. Wallace: He's Not Just Whistling Dixie

The presidential campaign of George C. Wallace, candidate of the American Independent Party, is an extraordinary operation which transfers many of the techniques and devices of Deep South provincial politics to the national scene. Wallace is making his forays into the north and west accompanied by a string band and a retinue of aides and advisers who are as southern as cornbread. And, by his verbal blasts against meddling by federal judges and "brief-case-totin' guideline-writers" in local school affairs, open housing, and union seniority lists, Wallace plays on racial fears—just as he did in 1963 when, in futile but symbolic protest against the admission of Negroes to the University of Alabama, he "stood in the schoolhouse door."

Blatant anti-intellectualism has been a feature of the Deep South politics of the Gene Talmadges and the Bilbos, and this, too, Wallace is using in his bid for the Presidency. Hardly a day goes by that Wallace fails to speak of "pointy-headed professors who can't park their bicycles straight." And, he tells his large and generally enthusiastic crowds, "the pseudo intellectuals look down their nose at the average man on the street, the steel worker, the auto worker, the textile worker, the fireman, the policeman, the barber, and the beautician."

Nor does he hesitate to suggest that university campuses are harboring disloyal and dangerous influences. When Wallace appeared in Cleveland recently, the hecklers, many of them of college age, were present in force, at times creating a formidable din. "You're paying taxes to support colleges and universities here in Ohio, but they seem to be teaching anarchy," Wallace said. Wallace invariably draws the line between what he considers legitimate and treasonable dissent, and he did so again in Cleveland. "When I become President," he said, "we are going to have our attorney general seek out these college professors who are making these speeches calling for communist victory [in Vietnam] and put them under a good jail."

Wallace is, however, making an attempt to adapt his campaign to some of the norms of national political life. In selecting his vice-presidential running mate, he did not choose an Orval Faubus of Arkansas or a Marvin Griffin of Georgia. He chose, instead, a national figure in General Curtis LeMay, retired Air Force chief of staff. Furthermore, Wallace has made public a party platform which, except for a few notable oversights (as in its failure to mention civil rights and arms control), touches on the full array of national questions, from environmental pollution to U.S. objectives in space. And even some of Wallace's sharpest critics concede that his formal statements on Vietnam and foreign policy have shown evidence of some restraint.

One may assume that none of the indigenous institutions of Wallace's home state of Alabama are closer to national attitudes and values than are its educational institutions, especially its colleges and universities. Accordingly, it may be particularly useful to examine Wallace's relations with higher education as one significant test of his credentials in his new role as a national politician. But, first, let's look at his personal and political origins.

Much has been made of Wallace's modest beginnings, but, although Wallace knew financial hardship as a youth, his family could not really have been placed among Alabama's poor white farmers and other have-nots. The Wallaces lived in Clio, a small community in southern Alabama, where George's grandfather was a respected country doctor who once ran successfully for a term as probate judge. Wallace's father (who had a history of chronic illness and died at the age of 40) was a failure at farming and most of his other ventures, but had flair enough for politics to win election as chairman of the county governing board. Wallace's mother was a high school music teacher.

This is the last of three articles on the presidential candidates.