A VIEW OF GENETICS.

By

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The Nobel Statutes of 1900 charge each prize-winner to give a public lecture in Stockholm within six months of Commemoration Day. That I have fully used this margin is not altogether ingenious, since it furnishes a pleasant occasion to revisit my many friends and colleagues in your beautiful city during its best season.

The charge might call for a historical account of past "studies on genetic recombination and organization of the genetic material in bacteria", studies in which I have enjoyed the companionship of many colleagues, above all my wife. However, this subject has been reviewed regularly (36, 37, 38, 41, 42, 45, 49, 54, 55, 58) and I hope you will share my own inclination to assume a more speculative task, to look at the context of contemporary science in which bacterial genetics can be better understood, and to scrutinize the future prospects of experimental genetics.

The dispersion of a Nobel award in the field of genetics symbolizes the convergent efforts of a world-wide community of investigators. That genetics should now be recognized is also timely — for its axial role in the conceptual structure of biology, and for its ripening yield for the theory and practice of medicine. However, experimental genetics is reaching its full powers in coalescence with biochemistry: in principle, each phenotype should eventually be denoted as an exact sequence of amino acids in protein (79) and the genotype as a corresponding sequence of nucleotides in DNA (a, 63). The precise demarcation of genetics from biochemistry is already futile: but when genetics has been fully reduced to its molecular foundations, it may continue to serve in the same relation as thermodynamics to mechanics (69). The coordination of so many adjacent sciences will be a cogent challenge to the intellectual powers of our successors.

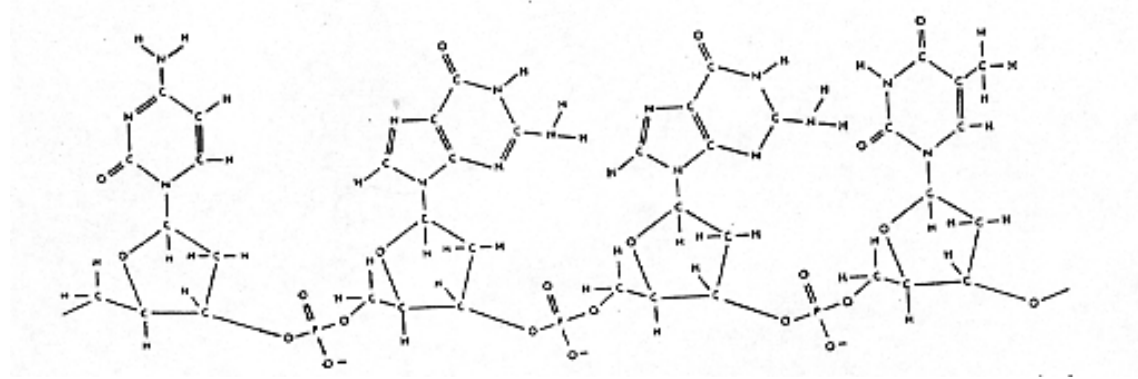a. No reader who recognizes *deoxyribonucleic acid* will need to be reminded what DNA stands for.

Fig. I. Primary structure of DNA — a segment of a polynuclotide sequence CGGT. From (13).

That bacteria and their genetics should now be so relevant to general biology is already a fresh cycle in our scientific outlook. When thought of at all, they have often been relegated to some obscure byway of evolution, their complexity and their homology with other organisms grossly underrated. "Since Pasteur's startling discoveries of the important role played by microbes in human affairs, microbiology as a science has always suffered from its eminent practical applications. By far the majority of the microbiological studies were undertaken to answer questions connected with the well-being of mankind" (30). The pedagogic cleavage of academic biology from medical education has helped sustain this distortion. Happily, the repatriation of bacteria and viruses is only the first measure of the repayment of medicine's debt to biology (6, 7, 8).

Comparative biochemistry has consummated the unification of biology revitalized by Darwin one hundred years ago. Throughout the living world we see a common set of structural units — amino acids, coenzymes, nucleins, carbohydrates and so forth — from which every organism builds itself. The same holds for the fundamental process of biosynthesis and of energy metabolism. The exceptions to this rule thus command special interest as meaningful tokens of biological individuality, *e. g.,* the replacement of cytosine by hydroxymethyl cytosine in the DNA of T2 phage (12).

Nutrition has been a special triumph. Bacteria which required no vitamins had seemed simpler than man. But deeper insights (32, 61) interpret nutritional simplicity as a greater power of synthesis. The requirements of more exacting organisms comprise just those metabolites they can not synthesize with their own enzymatic machinery.

Species differ in their nutrition: if species are delimited by their genes, then genes must control the biosynthetic steps which are

reflected in nutritional patterns. This syllogism, so evident once told, has been amplified by Beadle and Tatum from this podium. Its implications for experimental biology and medicine are well-known: among these, the methodology of bacterial genetics. Tatum has related how his early experience with bacterial nutrition reinforced the foundations of the biochemical genetics of Neurospora. Then, disregarding the common knowledge that bacteria were too simple to have genes. Tatum took courage to look for the genes that would indeed control bacterial nutrition. This conjunction marked the start of my own happy association with him, and with the fascinating challenges of bacterial genetics.

Contemporary genetic research is predicated on the role of DNA as the genetic material, of enzym3 proteins as the cell's working tools, and of RNA as the communication channel between them (63). Three lines of evidence substantiate the genetic function of DNA. Two are related to bacterial genetics; the third and most general is the cytochemical observation of DNA in the chromosomes, which are undeniably strings of genes. But chromosomes also contain other constituents besides DNA: we want a technique to isolate a chromosome or a fragment of one, to analyse it and to retransplant it to verify its functional capacity. The impressive achievements of nuclear transplantation (29) should encourage the audacity needed to try such experiments. The constructive equivalent to chromosome transplantation was discovered by a bacteriologist thirty years ago (20) but the genetic implications of the "pneu-mococcus transformation" in the minds of some of Griffith's successors were clouded by its involvement with the gummy outer capsule of the bacteria. However, by 1943, Avery and his colleagues had shown that this inherited trait was transmitted from one pneumococcal strain to another by DNA. The general transmission of other traits by the same mechanism (25) can only mean that DNA comprises the genes (b).

b. One might be tempted to write: "One DNA molecule = one gene." However, the quanta of factorial genetics, based on mutation, recombination and enzymatic function are all smaller than the DNA unit of molecular weight ~ 6 x 10^6 (4). There is increasing evidence that such a molecule is a natural unit rather than an artefact of fragmentation (64).

To reinforce this conclusion, HERSHEY and CHASE (23) proved that the genetic element of a bacterial virus is also DNA. Infection of a host cell requires the injection of just the DNA content of the adsorbed particle. This DNA controls not only its own replication in the production of new phage but also the specificity of the protein coat, which governs the serological and host range specificity of the intact phage.
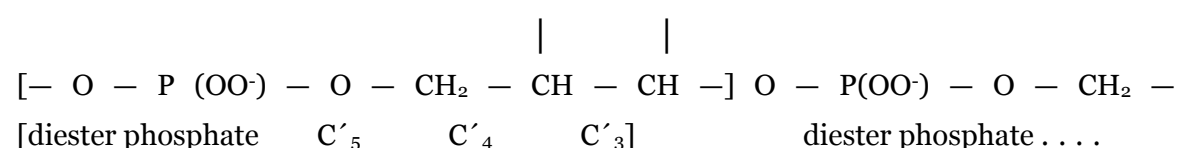
At least in some small viruses, RNA also displays genetic functions. However, the hereditary autonomy of gene-initiated RNA of the cytoplasm is now very doubtful — at least some of the plasmagenes that have been proposed as fulfilling this function are now better understood as feedback-regulated systems of substrate-transport (65, 72, 81).

The work of the past decade thus strongly supports the simple doctrine that genetic information is nucleic, *i. e.,* is coded in a linear sequence of nucleotides. This simplification of life may appear too facile, and has furnished a tempting target for agnostic criticism (37, 41, 44, 74). But, while no scientific theory would decry continual refinement and amplification, such criticism has little value if it detracts from the evident fruitfulness of the doctrine in experimental design.

The cell may, of course, carry information other than nucleic either in the cytoplasm or, accessory to the polynucleotide sequence, in the chromosomes. Epinucleic information has been invoked, without being more precisely defined, in many recent speculations on cytodifferentiation and on such models of this as antigenic phase variation in *Salmonella* (71, 52, 56, 47). Alternative schemes have so much less information capacity than the nucleic cycle that they are more likely to concern the regulation of genie functions than to mimic their specificities.

DNA as a Substance

The chemistry of DNA deserves to be exposed by apter craftsmen (86, 31, 13) and I shall merely recapitulate before addressing its biological implications. A segment of DNA is illustrated in Fig. 1. This shows a linear polymer whose backbone contains the repeating unit:

$$[- \text{O} - \text{P} (\text{OO}^-) - \text{O} - \text{CH}_2 - \overset{|}{\text{CH}} - \overset{|}{\text{CH}} -] \text{O} - \text{P}(\text{OO}^-) - \text{O} - \text{CH}_2 -$$

[diester phosphate C'_5 C'_4 C'_3]   diester phosphate . . . .

The carbon atoms are conventionally numbered according to their position in the furanose ring of deoxyribose, which is coupled as an N-glycoside to one of the nucleins: adenine, guanine, cytosine or thymine, symbolized A, G, C or T, the now well-known alphabet in which genetic instructions are composed. With a chain length of about 10 000 residues, one molecule of DNA contains 20 000 "bits of information", comparable to the text of this article, or in a page of newsprint.

Pyrophosphate-activated monomer units (*e. g.* thymidine triphosphate) have been identified as the metabolic precursors of DNA (31). For genetic replication, the monomer units must be assembled in a sequence that reflects that of the parent molecule. A plausible mechanism has been forwarded by WATSON

Fig. 2. The scheme of Watson and Crick for DNA replication. "Unwinding and replication proceed *pari passu.* All three arms of the Y rotate as indicated." (14).

and CRICK (87) as a corollary to their structural model whereby DNA occurs as a two-stranded helix with the bases being centrally oriented. When their relative positions are fixed by the deoxyribose-phosphate backbones, just two pairs of bases are able to form hydrogen bonds between their respective NH and CO groups; these are A: T and G: C. This pairing of bases would tie the two strands together for the length of the helix. In conformity with this model, extensive analytical evidence shows a remarkable equality of A with T and of G with C in DNA from

various sources. The two strands of any DNA are then mutually complementary, the A, T, G and C of one strand being represented by T, A, C and G, respectively, of the other. The information of one strand is therefore equivalent to, because fully determined by, the other. The determination occurs at the replication of one parent strand by the controlled stepwise accretion of monomers to form a complementary strand. At each step only the monomer which is complementary to the template would fit for a chain-lengthening esterification with the adjacent nucleotide. The model requires the unravelling of the intertwined helices to allow each of them to serve as a template. This might, however, occur gradually, with the growth of the daughter chain — a concept embedded in Fig. 2 which symbolizes the new Cabala. The discovery of a single-stranded configuration of DNA (85) makes complete unravelling more tenable as an alternative model.

For the vehicle of life's continuity, DNA may seem a remarkably undistinguished molecule. Its overall shape is controlled by the uniform deoxyribose phosphate backbone whose monotony then gives X-ray diffraction patterns of high crystallinity. The nucleins themselves are relatively unreactive, hardly different from one to the other, and in DNA introverted and mutually saturated. Nor are any of the hydroxyls of deoxyribose left unsubstituted in the polymer. The structure of DNA befits the solipsism of its function.

The most plausible function of DNA is ultimately to specify the amino acid sequence in proteins. However, as there are twenty amino acids to choose among, there cannot be a one: one correspondence of nucleotide to amino acid. Taking account of the code-duplication in complementary structures and the need to indicate spacing of the words in the code sequence, from three to four nucleins may be needed to spell one amino acid (19).

While a protein is also defined by the sequence of its monomeric units, the amino acids, the protein molecule lacks the "aperiodic crystallinity" (80) of DNA. The *differentiæ* of the amino acids vary widely in size, shape and ionic charge *(e. g.,* H •; CH_3 •; H_2N • CH_2 • CH_2 • CH_2 • CH_2 •; COOH • CH_2 • CH_2 •; HO • C_6H_4 • CH_2 •) and in the case of proline, bond angles. The biological action of a protein is, therefore, attributable to the shape of the critical surface into which the polypeptide chain folds

(73). The one-dimensional specificity of the DNA must therefore be translated into the three-dimensional specificity of an enzyme or antibody surface. The simplest assumption would be that the amino acid sequence of the extended polypeptide, as it is released from the protein-building template in the cytoplasm, fully determines the folding pattern of the complete protein, which may, of course, be stabilized by non-peptide linkages. If not we should have to interpose some accessory mechanism to govern the folding of the protein. This issue has reached a climax in speculations about the mechanism of antibody formation. If antibody globulins have a common sequence on which specificity is superimposed by directed folding, an antigen could directly mold the corresponding antibody. However, if sequence determines folding, it should in turn obey nucleic information. As this should be independent of antigenic instruction, we may look instead to a purely selective role of antigens to choose among nucleic alternatives which arise by spontaneous mutation (8, 50).

The correspondence between amino acids and clusters of nucleotides has no evident basis in their inherent chemical makeup and it now appears more probable that this *code* has evolved secondarily and arbitrarily to be translated by some biological intermediary. The coding relationship would then be analogous to, say, Morse-English (binary linear) to Chinese (pictographic). Encouragingly, several workers have reported the enzymatic reaction of amino acids with RNA fragments (22, 75). Apparently each amino acid has a different RNA receptor and an enzyme whose twofold specificity thus obviates any direct recognition of amino acid by polynucleotides. The alignment of amino-acyl residues for protein synthesis could then follow controlled assembly of their nucleotidates on an RNA template, by analogy with the model for DNA replication. We then visualize the following modes of information transfer:

(1) DNA replication — assembly of complementary deoxyribo-nucleotides on a DNA template.

(2) Transfer to RNA by some comparable mechanism of assembling ribonucleotides. Our understanding of this is limited by uncertainties of the structure of RNA (16).

(3) Protein synthesis:

    (a) Aminoacylation of polynucleotide fragments;

    (b) Assembly of the nucleotidates on an RNA template by analogy with step (1);

    (c) Peptide condensation of the amino acid residues.

Some workers have suggested that RNA is replicated in step (3) concurrently with protein synthesis, in addition to its initiation from DNA.

The chief difference in primary structure between DNA and RNA is the hydroxylation of C'_2 in the ribose, so that a reactive sugar hydroxyl is available in RNA. This may prove to be important in the less ordered secondary structure of RNA, and in its function as an intermediary to protein. It remains to be determined whether the aminoacyl nucleotidates are esterified at C'_2 or at C'_3 which is also available in the terminal residue. From this resume we may observe that the DNA backbone constitutes an inert but rigid framework on which the differential nucleins are strung. Their spatial constraint lends specificity to the pattern of hydrogen bonding exposed at each level. This extended pattern is a plausible basis for replication; it is difficult to visualize any reagents besides other nucleotides to which this pattern would be relevant. These conditions are quite apt for a memory device — rubber and guncotton are poor choices for a computing tape.

DNA and Bacterial Mutation

The *ignis fatuus* of genetics has been the specific mutagen, the reagent that would penetrate to a given gene, recognize and modify it in a specific way. Directed mutation has long been discredited for higher organisms and the "molar indeterminacy" of mutation established both for its spontaneous occurrence and its enhancement by X-rays (68). However, the development of resistance apparently induced by drugs revived illusions that bacterial genes might be alterable, an inference that would inevitably undermine the conception of "gene" for these organisms. No wonder that the mechanism of drug resistance has excited so much controversy (89)!

What sort of molecule could function as a specific mutagen, a reagent for a particular one of the bacterium's complement of genes, which can hardly number less than a thousand targets? On the nucleic hypothesis, the smallest segment capable of this variety would be a *hexa*nucleotide, all possible configurations of which must be discriminated by the specific mutagen. How could this be generally accomplished except by another molecule of conforming length and periodicity, that is an analogous polynucleotide? Certainly there is nothing in the chemistry of penicillin or streptomycin to support their direct intervention in nucleic instructions.

In addition, we recognize no chemical reagent capable of substituting one nuclein for another in the structure of existent DNA. However, as the modification of a nuclein, even to give an unnatural base, could have mutagenic effect, the chief limitation for specific mutagenesis is the recognition of the appropriate target.

Of course the origin of drug resistance, for all its theoretical implications, poses an experimental challenge of its own. Concededly, experiments cannot decide untried situations. Nevertheless, the mechanism whereby resistant mutants arise spontaneously and are then selected by the drug can account for every well-studied case of inherited resistance (5, 10). Furthermore, in favorable instances the spontaneous origin of drug-resistant mutants can be verified unambiguously by contriving to isolate them without their ever being exposed to the drug. One method entails indirect selection. To illustrate its application, consider a culture of *Escherichia coli* containing 10^9 bacteria per ml. By plating samples on agar containing streptomycin, we infer that one per million bacteria or 10^3 per ml produce resistant clones. But to count these clones they were selected in the presence of streptomycin which hypothetically might have induced the resistance. We may however dilute the original bacteria in plain broth to give samples containing 10^5 per ml. Since 10^{-6} of the bacteria are resistant, each sample has a mathematical expectation of 0,1 of including a resistant bacterium. The individual bacteria being indivisible by-dilution, nine samples in ten will include no resistants; the tenth will have one, but now augmented to 1 : 10^5. Which one this is can be readily determined by retrospective assay on the incubated samples. The procedure can be reiterated to enrich for

the resistant organisms until they are obtained in pure culture (11). The same result is reached more conveniently if we spread the original culture out on a nutrient agar plate rather than distribute samples into separate test tubes. Replica plating, transposing a pattern of surface growth from plate to plate with a sheet of velvet, takes the place of assaying inocula distributed in tubes (53). Dilution sampling and replica plating are then alternative methods of indirect selection whereby the test line is spared direct contact with the drug. Selection is accomplished by saving sublines whose *sibling* clones show the resistant reaction. This proof merely reinforces the incisive arguments that had already been forwarded by many other authors.

If mutations are not specific responses to the cellular environment, how do they arise? We still have very little information on the proximate causes of spontaneous, even of radiation and chemically induced mutation. Most mutagenic chemicals are potent alkylating agents *e. g.* formaldehyde or nitrogen mustard, which attack a variety of reactive groups in the cell. Similar compounds may occur in normal metabolism and account for part of the spontaneous mutation rate; they may also play a role as chemical intermediates in radiation effects. For the most part, then, studies on mutagenesis, especially by the more vigorous reagents, have told us little about the chemistry of the gene. Probably any agent which can penetrate to the chromosomes and have a localized chemical effect is capable of introducing random errors into the genetic information. If the cell were not first killed by other mechanisms most toxic agents would then probably be mutagenic.

Another class of mutagenic chemicals promises more information: analogues of the natural nucleins which are incorporated into DNA. For example, bromouracil specifically replaces thymine in phage DNA when furnished as bromodeoxyuridine to infected bacteria. FREESE has shown, by genetic analyses of the utmost refinement, that the loci of resulting mutations in T4 phage are distributed differently from the mutants of spontaneous origin or those induced by other chemicals (18). This method presumably maps the locations of thymine in the original DNA. In order to account for wide variations in mutation rate for different loci, further interactions among the nucleotides must be supposed. So far, these studies represent the closest approach to a rational basis for

chemical mutagenesis. However, every gene must present many targets to any nuclein analogue and the specificity of their mutagenesis can be detected only in systems where the resolution of genetic loci approximates the spacing of single nucleotides (4). At present this is feasible only in microorganisms; similar studies with bacteria and fungi would be of the greatest interest.

More specific effects might result from the insertion of oligo- and polynucleotides, a program which, however, faces a number of technical difficulties: even if the requisite polymers were to be synthesized, there are obstacles to their penetration into cells. The use of DNA extracted from mutant bacteria to transfer the corresponding genetic qualities is discussed as "genetic transduction".

RNA is the one other reagent that may be expected to recognize particular genes. As yet we have no direct evidence that the transfer of information from DNA to RNA is reversible. However, the anti-mutagenic effect of nuclein ribosides (21, 71) may implicate RNA in mutation. The reversibility of DNA ⇆ RNA information is also implicit in STENT's closely reasoned scheme for DNA replication (82). The needed experiment is the transfer of DNA information by some isolated RNA. Although not reported, this has probably not been fairly tried.

One motivation for this approach is the difficult problem of finding sources of homogeneous nucleic acids. DNA occurs biologically as sets of different molecules presumably in equimolar proportions. (A useful exception may be a remarkably small phage which seems to be unimolecular (85).) The species of RNA, however, may vary with the predominant metabolic activity of the cells. If so, some molecular species may be sufficiently exaggerated in specialized cells to facilitate their isolation. A purified RNA would have many potential applications, among others as a vehicle for the recognition of the corresponding DNA implied by our theory of information transfer. Pending such advances, *specific* mutagenesis is an implausible expectation.

Adaptive mutations, of which drug resistance is a familiar example, are crucial to the methodology of microbial genetics. Once having connected adaptive variation with gene mutation (78), we could proceed to exploit these systems for the detection of specific genotypes in very large test populations. The genotypes of interest may arise, as in the

previous examples, by mutation: the most extensive studies of the physiology of mutation now use these methods for precise assay. For, in

order to count the number of mutants of a given kind, it suffices to plate large numbers of bacteria into selective media and count the surviving colonies which appear after incubation. In this way, mutation rates as low as one per 10^9 divisions can be treated in routine fashion.

Genetic Recombination in Bacteria

The selective isolation of designed genotypes is also the most efficient way to detect genetic recombination. For example, the sexual mechanism of *Escherichia coli* was first exposed when prototrophic (nutritionally self-sufficient) recombinants arose in mixed cultures of two auxotrophic (nutritionally dependent) mutants (35, 84, 57). At first only one recombinant appeared per million parental bacteria and the selective procedure was quite obligatory. Later, more fertile strains were descovered which have been most helpful to further analysis (45, 51). This has shown that typical multinucleate vegetative bacteria unite by a conjugation bridge through which part or all of a male genome migrates into the female cell (43). The gametic cells then separate. The exconjugant male forms an unaltered clone, surviving by virtue of its remaining nuclei. The exconjugant female generates a mixed clone including recombinants (1, 46). WOLLMAN, JACOB and HAYES (88) have since demonstrated that the paternal chromosome migrates during fertilization in an orderly, progressive way. When fertilization is prematurely interrupted, the chromosome may be broken so that only anterior markers appear among the recombinants. All of the genetic markers are arranged in a single linkage group and their order can be established either by timing their passage during fertilization or by their statistical association with one another among the recombinants. Finally, the transfer of genetic markers can be correlated with the transfer of DNA as inferred from the lethal effect of the radioactive decay of incorporated p^{32} (27).

Sexual recombination is one of the methods for analysing the gene-enzyme relationship. The studies so far are fragmentary but they support the conception that the gene is a string of nucleotides which must function as a coherent unit in order to produce an active enzyme

(4, 15, 33, 67, 90). However, metabolic blocks may originate through interference with accessory regulatory mechanisms instead of the

fundamental capacity to produce the enzyme. For example, many "lactase-negative" mutants have an altered pattern of enzyme induction or a defective permease system for substrate-transport (55, 65). Several laboratories are now working to correlate the relative sequence of genetic defects with the sequence of corresponding alterations in enzyme proteins; this may be the next best approach to the coding problem short of a system where a pure DNA can be matched with its protein phenotype.

At first these recombination experiments were confined to a single strain of *E. coli,* K-12. For many purposes this is a favorable choice of material—perhaps the main advantage is the accumulation of a library of many thousands of substrains carrying the various markers called for by the design of genetic tests. However, strain K-12 is rather unsuitable for serological studies, having lost the characteristic surface antigens which are the basis of serological typing. In any event it would be important to know the breeding structure of the group of enteric bacteria. Systematic studies have therefore been made of the inter-fertility of different strains of bacteria, principally with a convenient tester of the K-12 strain (39, 93). About one fourth of the serotype strains of *E. coli* are fertile with strain K-12, and in at least some instances with one another. Whether the remaining three fourths of strains are completely sterile, or whether they include different, closed, breeding groups *(i. e.,* different genetic' species) has not been systematically tested, partly because of the preliminary work needed to establish suitable strains.

E. coli K-12 is also interfertile with a number of strains of Shigella spp. (59). Finally, although attempted crosses of *E. coli* with many *Salmonella* types and of *Salmonellas* with one another have usually failed, Baron has demonstrated crosses of *E. coli* with a unique strain of *Salmonella typhimurium* (3). This may be especially useful as a means of developing hybrids which can be used to bridge the studies of sexuality in *E. coli* and transduction in *Salmonella.*

Genes and Viruses

Bacteria furnish a unique opportunity to study the genetic relationships with their host cells. Another treasure of strain K-12 was for a time hidden, it carries a temperate phage, λ, which is technically quite favorable for genetic work. In accord with BURNET'S early predictions we had anticipated that the provirus for λ would behave as a genetic unit but Dr. ESTHER LEDERBERG'S first crosses were quite startling in their implication that the prophage segregated as a typical chromosomal marker (34). This was shown quite unambiguously by the segregation of lysogenicity versus sensitivity from persistent heterozygous cells, a test which bypassed the then controversial details of fertilization. The viability of such heterozygous cells supports the hypothesis that lysogenicity depends in part on the development of a cytoplasmic immunity to the cytopathic effects of infecting phage as a result of the establishment of the prophage in a bacterial chromosome. This picture is also brought out by "zygotic induction" (26), whereby the fertilization of a sensitive cell by a prophage-bearing chromosome may provoke the maturation and aggressive growth of the phage and lysis of the complex. On the other hand, the introduction of a sensitive chromosome into a lysogenic bacterium does not result in this induction. The mode of attachment of prophage to its chromosomal site is as unsettled as the general picture of the higher organization of DNA, but most students favor a lateral rather than an axial relationship for the prophage. The isolation of intact chromosomes of bacteria would give a new approach to this question but has so far been inconclusive.

Another infectious particle that has jumped out of our Pandora's box determines the very capacity of *E. coli* to function as a male partner in fertilization (51). For lack of a better inspiration, we call this particle "F". Two kinds of male strains are now recognized according to whether the F particle has a chromosomal or a cytoplasmic location. F+ strains, like the original K-12, are highly contagious for F and will rapidly convert populations of female, F— strains in which they are introduced. Hfr males, on the other hand, have a chromosomal localization of the F factor resulting from occasional transpositions in F+ strains. The different localization of the F particle in the two cases is diagnosed primarily by the behavior of the particle in crosses. In addition, HIROTA

and IIJIMA (24) found that the F particle could be eliminated from F+ strains by treatment with acridine dyes. Hfr clones are unaffected by acridine orange, but when they revert to the F+ state, as occasionally happens, the F particle again becomes vulnerable to the dye. The accessibility of extrachromosomal F is paralleled by several other examples of plasmid disinfection (reviewed in 40); perhaps the most notable is the bleaching of green plant cells by streptomycin (17, 76). No reagent is known to inactivate F or prophage while bound to the chromosome.

The virus λ and the plasmagene F are analogous in many features (28, 48). Their main differences are:

(1) Cytopathogenicity. A bacterium cannot long tolerate λ in its cytoplasmic state and remain viable. The vegetative λ must promptly reduce itself to a chromosomal state or multiply aggressively and lyse the host bacterium. F has no known cytopathic effect.

(2) Maturation. Vegetative λ organizes a protein coat and matures into an infective phage particle. F is known only as an intracellular vegetative element; however, the coat of the F+ cell may be analogous to that of the phage.

(3) Transmission. λ is infective, *i. e.,* forms a free particle which can penetrate susceptible cells. F is transmitted only by cell-to-cell conjugation.

(4) Fixation. λ has a fore-ordained site of fixation on the bacterial chromosome; F has been identified at a variety of sites. However, this difference may be illusory. In special situations, F does have preferential sites of fixation (77), and generally, translocations of F to different sites are more readily discovered than those of λ would be.

(5) Induction. Exposure of lysogenic bacteria to small doses of ultra-violet light causes the prophage to initiate a lytic cycle with the appearance first of vegetative, then of mature phage (62). Hfr bacteria make no analogous response. However, the kinetics of the reversion, Hfr → F+, has not been carefully studied.

The genetic function of bacteriophages is further exemplified by *transduction* whereby genes are transferred from cell to cell by the intervention of phage particles (42, 91). In our first studies we

concluded that the bacterial genes were adventitiously carried in normal phage particles (66, 83, 92). Further studies favor the view that the transducing particle has a normal phage coat but a *defective* phage nucleus. This correlation has suggested that a gene becomes transducible when a prophage segment is translocated to its vicinity (2, 9, 60).

Transduction focuses special attention on the phenomenon of specific pairing of homologous chromosome segments. Howsoever a transduced gene is finally integrated into the bacterial genome, at some stage it must locate the homologous gene in the recipient chromosome. For in transduction, as in sexual recombination, new information is not merely added to the complement; it must also replace the old. This must involve the confrontation of the two homologues prior to the decision which one is to be retained. Synapsis is even more, puzzling as between chromosomes whose DNA is in the stabilized double helix and then further contracted by supercoiling. Conceivably gene products rather than DNA are the agency of synaptic pairing.

The integration of a transduced fragment raises further issues (41). The competing hypotheses are the physical incorporation of the fragment in the recipient chromosome, or the use of its information when new DNA is replicated. The same issues still confound models of crossing over at meiosis in higher forms; once again the fundamentals of chromosome structure are needed for a resolution.

Virus Versus Gene

The homology of gene and virus in their fundamental aspects makes' their overt differences even more puzzling. According to the simplest nucleic doctrine, DNA plays no active role in its own replication other than furnishing a useful pattern. Various nucleotide sequences should then be equally replicable. What then distinguishes virus DNA, which replicates itself at the expense of the other pathways of cellular anabolism? For the T even phages, the presence of the unique glucosylated hydroxymethylcytosine furnishes a partial answer (12). However, other viruses such as λ display no unique constituents; furthermore, as prophage they replicate coordinately with bacterial

DNA. Does the virus have a unique element of structure, either chemical or physical, so far undetected? Or does it instruct its own preferential synthesis by a code for supporting enzymes?

The Creation of Life

The mutualism of DNA, RNA and proteins as just reviewed is fundamental to all contemporary life. Viruses are simpler as infective particles but must, of course, parasitize the metabolic machinery of the host cell. What would be the least requirements of a primeval organism, the simplest starting point for progressive replication of DNA in terms of presently known or conjectured mechanisms? They include at least:

(1) DNA

(2) The four deoxyribotide pyprophosphates in abundance.

(3) One molecule of the protein, DNA polymerase.

(4) Ribotide phosphates as precursors for RNA.

(5) One molecule of the protein RNA polymerase.

(6) A supply of the twenty amino acyl nucleotidates.

  (a) Failing these, each of the twenty enzymes which catalyse the condensation of an amino acid and corresponding RNA fragments together with sources of these components.

(7) One molecule of the protein aminoacyl-RNA polymerase.

In principle, this formidable list might be reduced to a single polynucleotide polymerized by a single enzyme. However, any scheme for the enzymatic synthesis of nucleic acid calls for the coincidence of a particular nucleic acid and of a particular protein. This is a far more stringent improbability than the sudden emergence of an isolated DNA such as many authors have suggested, so much more so that we must look for alternative solutions to the problem of the origin of life. These are of two kinds. The primeval organism could still be a nucleic cycle if nucleic replication occurs, however imperfectly, without the intervention of protein. The polymerase enzyme, and the transfer of information from nucleic acid to protein, would then be evolved refinements. Alternatively, DNA has evolved from a simpler, spontaneously condensing polymer. The exquisite perfection of DNA makes the second suggestion all the more plausible.

The nucleoprotein cycle is the climax of biochemical evolution. Its antiquity is shown by its adoption by all phyla. Having persisted for $\sim$ 10^9 years, nucleoprotein may be the most durable feature of the geochemistry of this planet.

At the present time, no other self-replicating polymers are known or understood. Nevertheless, the nucleic system illustrates the basic requirements for such a polymer. It must have a rigid periodic structure in which two or more alternative units can be readily substituted. It must allow for the reversible sorption of specific monomers to the units in its own sequence. Adjacent, sorbed monomers must then condense to form the replica polymer, which must be able to desorb from the template. Primitively, the condensation must be spontaneous but reliable. In DNA, the sorption depends on the hydrogen bonding of nuclein molecules constrained on a rigid helical backbone. This highly specific but subtle design would be difficult to imitate. For the more primitive stages, both of biological evolution and of our own experimental insight, we may prefer to invoke somewhat cruder techniques of complementary attachment. The simplest of these is perhaps the attraction between ionic groups of opposite charge, for example, NH_3^+ and COO^- which are so prevalent in simple, organic compounds. If the ingenuity and craftmanship so successfully directed at the fabrication of organic polymers for the practical needs of mankind were to be concentrated on the problem of constructing a self-replicating assembly along these lines I predict that the construction of an artificial molecule having the essential function of primitive life would fall within the grasp of our current knowledge of organic chemistry.

Conclusions

The experimental control of cellular genotype is one of the measures of the scope of genetic science. However, nucleic genes will not be readily approached for experimental manipulation except by reagents that mimic them in periodic structure. Specifically induced mutation, if ever accomplished, will then consist of an act of genetic recombination between the target DNA and the controlled information specified by the reagent. Methods for the step-wise analysis and re-assembly of nucleic

acids are likely to be perfected in the near future in pace with the accessibility of nucleic acid preparations which are homogeneous enough to make their use worth-while. For the immediate future, it is likely that the greatest success will attend the use of biological reagents to furnish the selectivity needed to discriminate one among innumerable classes of polynucleotides. Synthetic chemistry is, however, challenged to produce model polymers that can emulate the essential features of genetic systems.

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