Meetings

Molecular Basis of Heredity

The molecular basis of infectious heredity was the main subject of a symposium held under the auspices of the United States–Japan Cooperative Science Program in Honolulu, Hawaii, 4–7 May 1965. The Cooperative Program stems from a government-to-government agreement to promote cooperation in science between the two countries and is administered by the Japan Society for the Promotion of Science and the National Science Foundation.

A major topic of discussion was the nature and function of those genetic elements that control transfers of genetic material between intact bacterial cells. These elements may be called "conjugons." Three classes of such conjugons are known: the F or fertility factors, the R or antibiotic-resistance factors, and the *col* or colicinogenic factors. It was pointed out by E. A. Adelberg (Yale University) that in the light of current ideas on genetic transfers a conjugon must have one or more of the following three functions: (i) promotion of bridge formation between paired cells; (ii) initiation of DNA replication; (iii) ability to recombine with the bacterial chromosome and to promote its transfer. Several of the scientists reported advances on this subject. L. S. Baron (Walter Reed Army Hospital) reported on work performed by him and his co-workers on the recognition of the DNA of the conjugons by density gradient centrifugation. This kind of analysis has made it possible to recognize the presence or absence of genetic material homologous to the conjugons in different bacteria. Baron, as well as Y. Hirota (Osaka University), S. Mitsuhashi (Gunma University), and T. Watanabe (Kyoto University), reported experiments that indicate that both the R and F factors may become defective by mutations that suppress their ability to cause bridge formation. Complementation between defective R and F factors has been observed. Recombination can occur between these two classes of factors. Likewise, it appears that F factors can become associated with colicinogenic factors to form complex genetic elements, by a mechanism that may not be basically different from that which gives origin to transducing bacteriophages.

In connection with the relation of DNA synthesis to genetic transfer, Adelberg reported that nalidixic acid (NDX), an agent which selectively inhibits DNA synthesis, specifically blocks genetic transfer. T. Yura (Kyoto University) obtained mutations to resistance to phenethyl alcohol, another inhibitor of DNA synthesis, and showed that such mutation can occur either in the F factor or in the chromosomal structure of *Escherichia coli*. H. Ozeki (National Institute of Health, Tokyo) discussed studies on *col*E_2 factor, which is transmitted passively by bacteria which are also transferring the conjugon *col*I. His experiments indicate that the DNA of *col*E_2 need not be replicated in the course of genetic transfer. Thus, it appears that only the DNA which is physically associated with the conjugon itself is usually replicated during genetic transfer. Ozeki also discussed experiments on the mechanism that makes cells of *Salmonella typhimurium* competent to give high-frequency genetic transfer soon after having received the *col*I conjugon by contact. After a few generations, the "competence machinery," clearly a gene product, is not produced any more but remains intact in a fixed number of bacterial cells, which transmit it unilinearly to one descendant at each cell division. This persistent mechanism for genetic transfer is probably a local alteration of a surface structure, which, however, can still be regenerated after being destroyed by periodate.

In connection with genetic transfer, H. Uchida (University of Tokyo) and T. Watanabe (Keio University) discussed the *pili* or *fimbriae*, proteinaceous appendages of bacteria, one class of which, the F pili, has recently been shown to be correlated with genetic transfer in *E. coli*. The possible role of pili in genetic transfer mediated by R or *col* factors is still uncertain.

A second set of topics concerned phenomena observed with temperate phages, including prophage integration, transduction, and the mechanism of immunity. S. E. Luria (Massachusetts Institute of Technology) and A. Campbell (University of Rochester, discussed experiments from their own and other laboratories, which confirm the linear integration of prophage λ and related prophages, such as $\phi 80$, within the bacterial chromosome. Evidence to this effect comes from the linear pattern of cotransduction of phage genes with bacterial genes, from the linear pattern of deletions that remove congruent segments of prophage and bacterial genes, and from experiments on mixed infection with two phages leading to double lysogeny. The linear integration of prophage into the host chromosome, involving apparently a phase of circularization of the phage genome prior to integration as originally proposed by Campbell, accounts for the origin of a series of transducing phages from the integrated prophages.

J. Tomizawa (National Institute of Health, Tokyo) reported experiments on transduction by bacteriophage P1, which indicate that the majority of the transduced fragments of genetic material are segments of bacterial DNA synthesized before infection with the transducing phage. This is true only of chromosomal fragments since R and F elements, when transduced by phage P1, apparently do multiply before being enclosed in the transducing phage particles. Tomizawa's finding on chromosomal fragments indicate that Luria's earlier suggestion, that transduction may be mediated by composite genetic fragments containing phage and bacterial genes, is not generally valid. S. Mitsuhashi (Gunma University) described a new instance in which phage P1 becomes stably associated with a genetic marker to give a high-frequency transducing phage, which carries the chloramphenicol-resistance gene derived from an R factor. Thus, it appears that phage P1 can act in transduction both as a passive vehicle and as a source of persistent joint transducing elements. A remarkable finding by Tomizawa is that the chromosomal fragments in phage P1 are apparently tightly bound with a polypeptide whose function remains unknown.

According to Tomizawa, the length of the transduced fragment of bacterial DNA is determined by the actual size

of the transducing phage particle and is smaller in those P1 particles that have "pinhead" shape. The possibility that the amount of DNA enclosed in a phage particle is always determined by the process of capsid formation, as originally suggested by G. Streisinger (University of Oregon), was also supported by other evidence. The problem of uniformity of the terminals of the transduced genetic fragments, originally proposed by Ozeki, was discussed by Luria on the basis of experiments by J. Rothman which suggest that several overlapping classes of fragments can be present in transducing particles.

T. Horiuchi (National Institute of Health, Tokyo), J. Tomizawa, and H. Uchida reported experiments on the phage λ immunity repressor, which is supposedly responsible for blocking the expression of most phage functions in lysogenic bacteria. Using a class of mutants which produces a temperature-sensitive repressor, it has been found that the repressor can persist and remain functional for several generations after the repressor-producing gene has been eliminated from the cell. An estimate of 20 to 50 units of immunity repressor per cell in the steady state was arrived at. This estimate agrees with previous estimates by different methods.

In connection with repression phenomena, T. Iino (National Institute of Genetics, Misima) reported recent experiments on the mechanisms of regulation that control the alternative expression of phase I and phase II flagellar genes in *Salmonella*. Hirota, Y. Ikeda (University of Tokyo), and A. Tsugita (Osaka University) reported experiments on a complex regulatory mechanism that controls the production of alkaline phosphatase and phosphodiesterase in *Bacillus subtilis*.

Several presentations were devoted to the analysis of events that take place in the lytic cycle of bacteriophage growth, including recombination, maturation, and the regulation of the sequential events in the phage cycle. Tomizawa presented experiments which indicate that recombination in phage T4 gives rise at first to recombinant genetic molecules whose original moieties are held together by hydrogen bonds only. Later in the course of infection these recombinant molecules become bound by covalent bonds, presumably phosphodiester bonds. By the use of various mutants and inhibitors it can be shown that both the formation of the hydrogen-bonded recombinants and their conversion into covalently bonded forms require protein synthesis after infection by phage. In vitro experiments suggest that an enzyme formed after phage infection (possibly the DNA polymerase synthesized under the control of cistron 43?) is operative in the process of covalent bond formation. The actual nature of the interaction of phage DNA molecules to form recombinants, whether an end-to-end interaction or end-to-middle attack, was discussed but the evidence is not yet conclusive.

The maturation mechanism of phage T4 was discussed by R. S. Edgar (California Institute of Technology), who reported the existence of a mutant with only two-thirds as much DNA as the normal phage and a correspondingly smaller capsid. The probable relation of capsid formation to DNA size determination, already mentioned in connection with transduction by phage P1, is also supported by the reported finding that "condensation" of T4 phage DNA is not seen in sections of bacteria infected with T4 mutants that cannot synthesize the main capsid protein (phage head protein). In this connection, N. Zinder (Rockefeller Institute) reported that, with the RNA phage f2, the mutants which produce a capsid protein incapable of becoming assembled into normal capsids continue to accumulate RNA replicase and double-stranded replicative forms of the phage genome. It appears that in infection with this phage the two sets of processes, replication and enzyme formation versus maturation, are competitive with one another.

On the basis of studies of mixed infection between phages λ and $\phi 80$, Ozeki reported that the specific size of the capsid, measured by the density of the phage particles, appears to be associated univocally with the host range property, which is presumably determined by one of the tail proteins of the phage. This suggests that only one type of capsid assembly is possible, in the sense that head protein and tail proteins corresponding to two different phages cannot blend into one capsid.

The problem of regulatory events in the bacteriophage cycle was discussed by Edgar and Streisinger. Edgar presented the latest version of the genetic map of bacteriophage T4 and discussed several classes of genes whose mutations result in altered regulation of the sequential expression of phage functions. Some of the genes appear to regulate the time of initiation of synthesis of DNA and capsid proteins. Some other genes are required for the initiation of DNA synthesis and for the arrest of the early functions of the bacteriophage. Another class of genes allows formation of DNA but no late proteins, suggesting an inability to transcribe or translate the message for these proteins. Finally, other regulatory genes lead to a precocious arrest of all synthesis in infected cells by a too-early expression of a mechanism which operates normally just before lysis.

The occurrence of regulatory genes in phage is also shown by the existence of a cistron whose mutations reduce the level of all the three antigens that are part of the tail fibers and which are made by three separate cistrons.

G. Streisinger reported experiments that suggest that the messenger for the early phage functions made after phage infection is a relatively stable messenger whose protein continues to be made for at least 20 or 30 minutes. These experiments also indicate that some of the messenger for late functions may be made relatively early after phage infection but expressed only much later.

A topic on which major advances were reported is that of the nature and operation of the genetic code. Collaborative experiments by G. Streisinger, A. Tsugita, and their co-workers have revealed the presence of a sequence of five altered amino acids in the lytic enzyme (lysozyme) produced by a double mutant of bacteriophage T4 which carries two mutually compensating, proflavine-induced mutations separated by approximately 0.01 recombination units. This experiment is a direct confirmation of the theory, formulated by Crick and Brenner in 1961 on the basis of studies on proflavine-induced phage mutations, of the triplet nature of the genetic code and the directional mechanism of genetic transcription. The experiments reported by Streisinger and Tsugita represent the biochemical verification of the strongest inference from Crick and Brenner's theory of gene function. In addition, an analysis of the amino acid substitution observed in the mutant lysozyme, on the basis of the codon assignments by Nirenberg and other workers, has led to the conclusion that the translation of the RNA message into protein proceeds from the 5′-hydroxyl end to the 3′-end of the RNA.

G. Stent (University of California, Berkeley) summarized evidence that suggests that the transcription of the

messenger RNA on the DNA template also occurs from the 5′- to the 3′-end, and discussed possible mechanisms by which translation and transcription interact at the DNA site.

T. Yura discussed the present status of experiments in his laboratory in which the synthesis of the enzyme tryptophan synthetase by cell-free extract is stimulated by DNA carrying the corresponding genes and is subject to specific regulatory effects, thus suggesting that the in vitro system reproduces faithfully the machinery presumed to be operative in vivo.

A. Garen (Yale University) presented a series of experiments on the effect of suppressor mutations on the translation of the genetic message for the enzyme alkaline phosphatase. These studies, as well as parallel ones in other laboratories, have made it possible to identify the nature of at least one "nonsense" codon in *E. coli* RNA as A.U.G. and to identify the nature of the amino acid replacements caused by various suppressors. Garen and Zinder also reported current experiments which attempt to make use of genetic suppressors to induce specific changes in protein synthesized in cell extracts and to identify the mode of action of the suppressors, whether on the sRNA, the activating enzymes, or the ribosomes.

One significant conclusion to be derived from the valuable exchange of information that took place at the symposium is the close similarity of goals and methodology employed by scientists in the two countries, a similarity not surprising in view of the fact that many of the Japanese workers in molecular genetics have received part of their training in American laboratories. At the same time, the emphasis is often different and the discussion, therefore, was more mutually informative than a similar discussion would have been among a group of American scientists. In part, this is due to the fact that the Japanese molecular geneticists are often in closer touch with medical research than their American counterparts, and also reflects the fact that the interest of many Japanese scientists has been concentrated on certain phenomena, such as the resistance transfer factors, which have been discovered in Japan.

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Forthcoming Events

October

7–8. **Fiber** Soc., meeting, Wilmington, Del. (Fiber Soc., Box 625, Princeton, N.J.)

7–9. **Seismological** Soc. of America, eastern sec. 37th annual, Lamont Geological Observatory, Palisades, N.Y. (J. Dorman, Lamont Geological Observatory, Palisades 10964)

8–9. **Atlantic Coastal Plain Geological** Assoc., field trip, South Carolina. (D. J. Colquhoun, Dept. of Geology, Univ. of South Carolina, Columbia)

8–9. **Indiana** Acad. of Science, fall meeting, Notre Dame. (C. F. Dineen, St. Mary's College, Notre Dame)

9. **Paleontological Research** Inst., Ithaca, N.Y. (K. V. W. Palmer, Paleontological Research Inst., 109 Dearborn Pl., Ithaca)

9–10. **Gastroenterology**, French conf., Paris, France. (R. Biguie, 79, Boulevard Malesherbes, Paris 8°)

9–13. American Soc. of **Clinical Hypnosis**, Chicago, Ill. (F. D. Nowlin, ASCH, 800 Washington Ave., SE, Minneapolis, Minn. 55414)

9–17. Electrical, Electronics, and Mechanical **Engineering**, first Pan American congr., Mexico, D.F. (Inst. of Electrical and Electronics Engineers, Box A, Lenox Hill Station, New York 10021)

10–14. **Water Pollution Control** Fed., 38th annual, Atlantic City, N.J. (R. E. Fuhrman, 4435 Wisconsin Ave., NW, Washington, D.C. 20016)

10–15. International Federation for **Documentation**, congr., Washington, D.C. (Secretariat, FID, 9650 Wisconsin Ave., Washington 20014)

10–15. **Electrochemical** Soc., meeting, Buffalo, N.Y. (Executive Secretary, ES, 30 E. 42 St., New York 10017)

10–15. **Endocrinology**, 6th Pan American conf., Mexico, D.F. (G. Gual, Inst. Nacional de la Nutrición, Dr. Jimenez No. 261, Mexico 7)

10–16. American **Documentation** Inst., Washington, D.C. (J. E. Bryan, 2000 P St., NW, Washington, D.C. 20036)

10–17. **Bronchoesophagology**, 1st Latin American congr., Rio de Janeiro, Brazil. (F. Aprigliano, Rua Alcindo Guanabara, 24, Sob-Loja 206, Rio de Janeiro)

10–17. **Otorhinolaryngology**, 14th Brazilian congr., Rio de Janeiro, Brazil. (W. Benevides, Rua Alcindo Guanabara, 24, Sob-Loja 206, Rio de Janeiro)

10–17. **Plastic Surgery**, 10th Latin American congr., Buenos Aires, Argentina. (J. Norberto Spera, Riglos 624, Buenos Aires)

11–13. **Color Centers in Alkali Halides**, symp., Univ. of Illinois, Urbana. (D. W. Compton, Dept. of Physics, Univ. of Illinois, Urbana)

11–13. **Communications**, 11th natl. symp., Utica, N.Y. [G. E. Brunette, Communications Div. (EMCT) Rome Air Development Center, Griffiss AFB, New York 13442]

11–13. Metabolic Roles of **Lipids**, symp., Cincinnati, Ohio. (C. H. Hauber, American Oil Chemists' Soc., 35 East Wacker Dr., Chicago 1, Ill.)

11–13. **Manned Spaceflight**, 4th meeting, St. Louis, Mo. (J. F. Yardley, McDon-