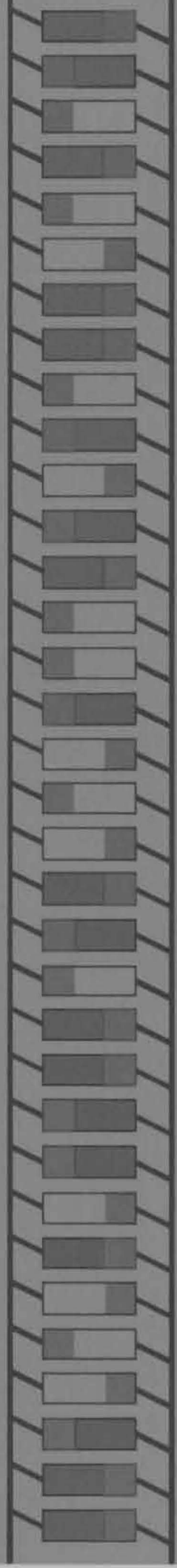
Annual Report of the Biology Division
1967

Southwest Center for Advanced Studies



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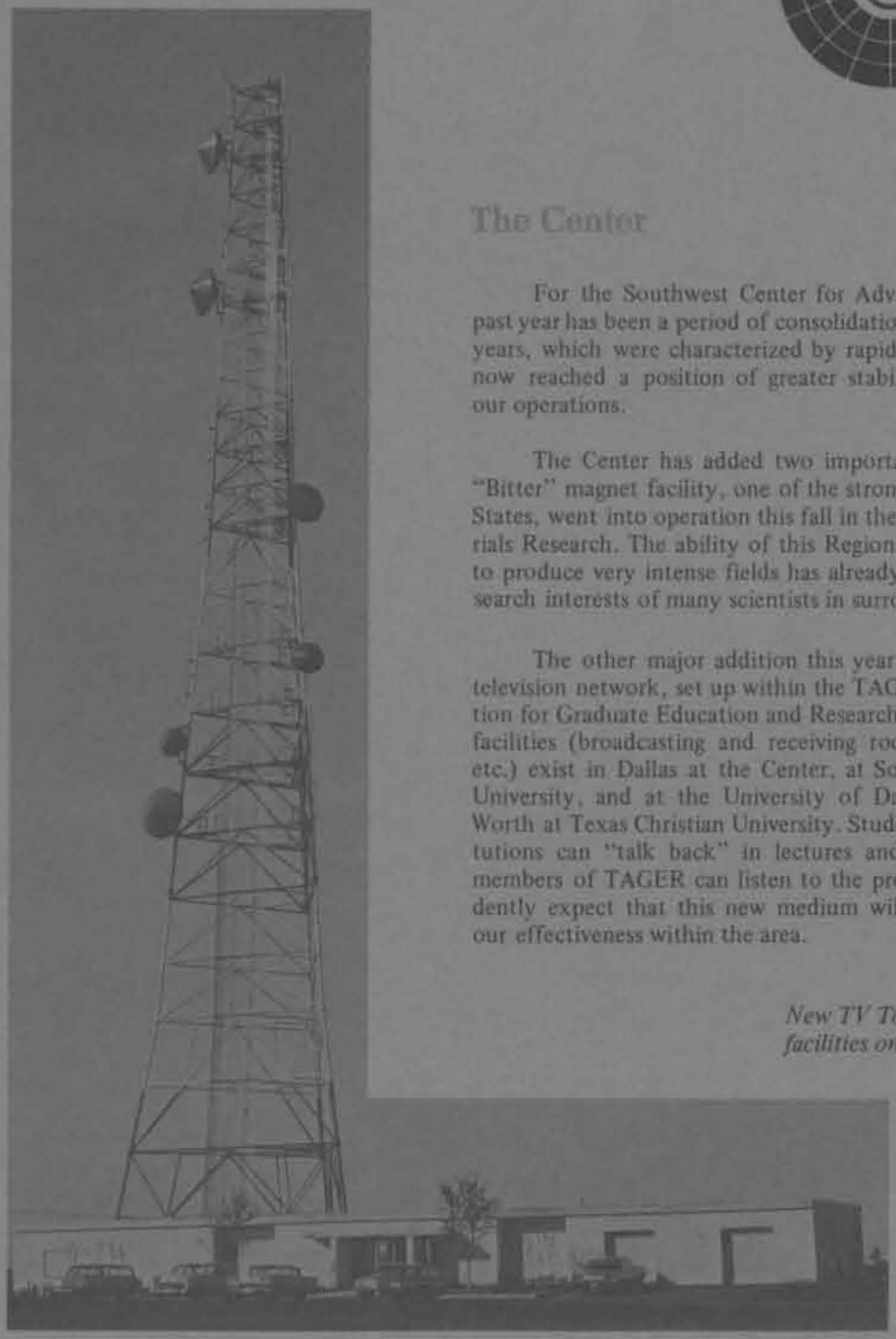


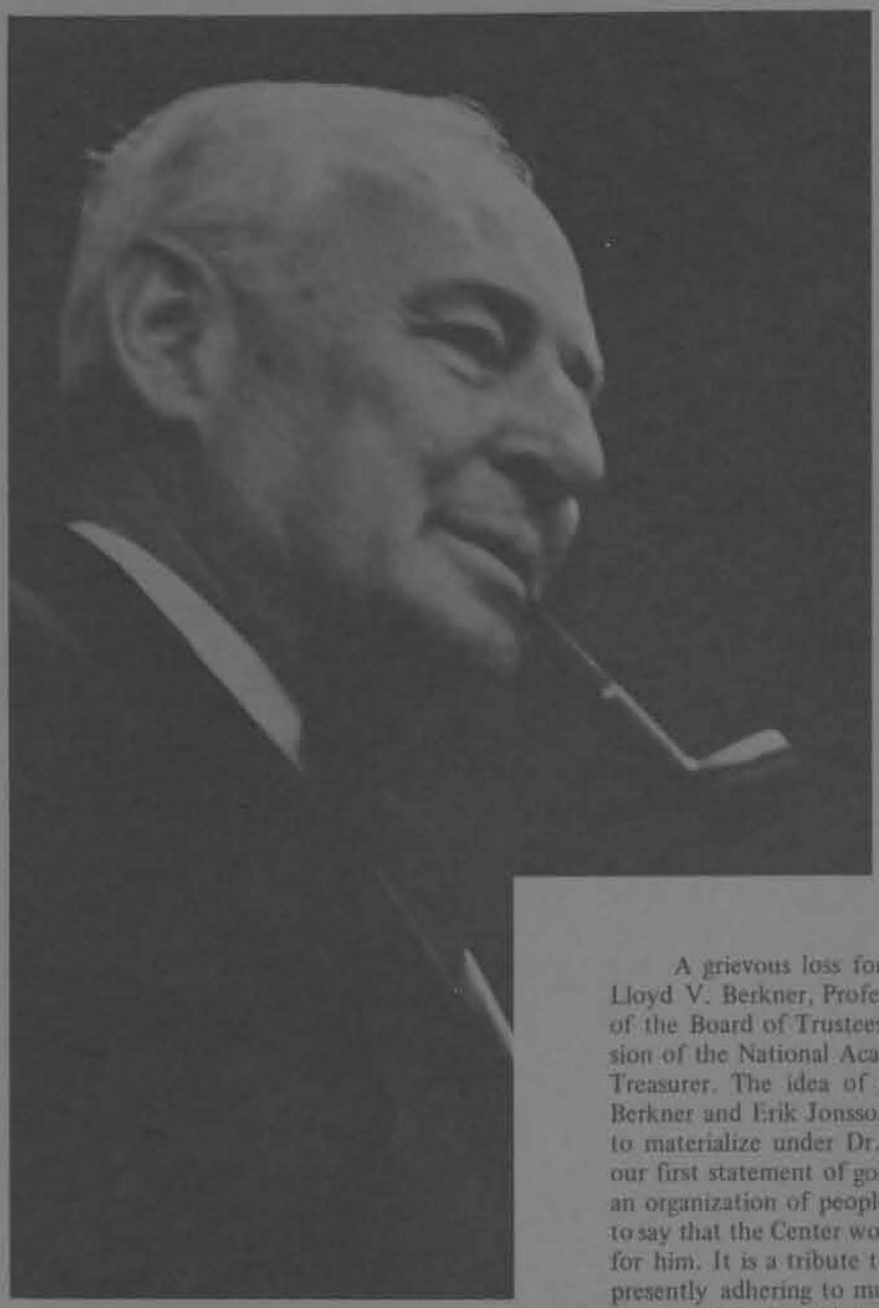
The Center

For the Southwest Center for Advanced Studies the past year has been a period of consolidation, unlike previous years, which were characterized by rapid growth. We have now reached a position of greater stability and depth in our operations.

The Center has added two important facilities. The "Bitter" magnet facility, one of the strongest in the United States, went into operation this fall in the Division of Materials Research. The ability of this Regional Magnet Facility to produce very intense fields has already attracted the research interests of many scientists in surrounding institutes.

The other major addition this year is an educational television network, set up within the TAGER (The Association for Graduate Education and Research) system. Primary facilities (broadcasting and receiving rooms, transmitters, etc.) exist in Dallas at the Center, at Southern Methodist University, and at the University of Dallas, and in Fort Worth at Texas Christian University. Students at these institutions can "talk back" in lectures and seminars. Other members of TAGER can listen to the programs. We confidently expect that this new medium will greatly increase our effectiveness within the area.

New TV Tower and TAGER facilities on SCAS campus



LLOYD V. BERKNER
1905–1967

A grievous loss for the Center this year was that of Lloyd V. Berkner, Professor of Geosciences and Chairman of the Board of Trustees, who died last June during a session of the National Academy of Sciences, of which he was Treasurer. The idea of our Center was conceived by Dr. Berkner and Erik Jonsson, the Mayor of Dallas, and began to materialize under Dr. Berkner in 1961. He established our first statement of goals and crystallized these ideas into an organization of people, buildings, and activities. It is fair to say that the Center would not exist today had it not been for him. It is a tribute to his vision that we find ourselves presently adhering to much the same pattern of objectives that he originally laid down.

In his activities, Dr. Berkner was assisted by a man with a long established academic career: Dr. Lauriston C. Marshall, former Director of the Office of Scientific Personnel at the Center. "Larry" Marshall retired this summer from his later position as Head of the Division of Materials Research, and is now associated with the University of Southern Illinois. The Biology Division remembers him with affection and expresses gratitude for the crucial role he played in the early days of our Division.

The Biology Division

The administrative responsibilities of Dr. Bresch, as Division Head, are now being shared with two Section Heads, Drs. Clowes and Jagger, with the assistance of Mrs. Thweatt and Mrs. Mormino. As in previous years, responsibility for all scientific facilities of the Division has remained in the able hands of Dr. Wiemann. Dr. Harris has been in charge of Divisional educational activities in general, while Dr. Haefner has carried the special responsibility for Divisional television.

The Biology Division has maintained a faculty size of around 20. Assistant Professors Myer Coval and Edwin Horowitz left our faculty at the end of the year, and Associate Professor Wolfram Heumann left in the fall to assume a chair in microbiology at the University of Erlangen, Germany. These losses are balanced by the arrival of Associate Professor Yvonne Lanni (our first female faculty member), formerly of Emory University Medical School, and Assistant Professor Philip Witonsky, from Dr. Khorana's laboratory at the University of Wisconsin, as well as by the promotion of Dr. Hermann Bujard to the rank of Assistant Professor. We are pleased to have had in our midst as Visiting Professor during the past six months an authority on genetic recombination, Harold L. K. Whitehouse of the University of Cambridge.

We have had a small increase in space in the Division this year, and have added several major items of equipment, such as an amino acid analyzer, an infrared spectrophotometer, and spectrofluorimeter.

At the beginning of the year, we had two Career Development Awards (NIH), twelve individual research grants (NIH, NSF), and one large Divisional research grant (NIH). During the year, three of these awards expired and all three were renewed. In addition, Dr. Bauerle was given a Career Development Award, and we have received five more individual research grants (NIH, NSF, AEC), and one general research grant (NIH). We thus presently have a total of three Career Development Awards, seventeen individual research grants, and two Divisional grants.

Yvonne T. Lanni

Philip Witonsky

Our various educational activities have continued. Our third annual summer program for college undergraduates, organized by Dr. Patrick, was held again this year, with 25 students participating. In these programs, the students work on limited research projects in the laboratories, and are given a special series of lectures, in addition to having access to the usual faculty seminars and research discussions. At the end of their 10-week stay, they are required to present both oral and written reports.

We continue to have a small number of graduate students in the Division. These people have completed their course work and are in residence at the Center to complete their thesis research, whereupon they will receive their degrees from their home institutions. The number of post-doctoral fellows has increased to sixteen.

Drs. Jagger and Rupert have become members of the graduate faculty committee of the Radiology Department of the University of Texas Southwestern Medical School in Dallas, where they are helping in a growing graduate program in Radiation Biology. Within this program, Dr. Rupert, with the aid of Drs. Harm and Jagger, presented a course last spring on Ultraviolet Photobiology. At the same institution, a course in Molecular Genetics was given in the Department of Anatomy by Drs. Bauerle, Bujard, Clowes, and Krone. Many of our faculty hold adjunct appointments in the Medical School.

Our really new effort in education this year has, of course, involved the TAGER TV network, on which a first biology course, *Topics in Physiology*, was presented in the fall by Dr. Harris. In addition, many biology seminars, of both outside and Divisional speakers, have been telecast. We look forward to extended and varied activities in this new educational medium.

John Jagger, Royston C. Clowes, and Carsten Bresch preparing this Annual Report

R. BAUERLE, D. SMITH

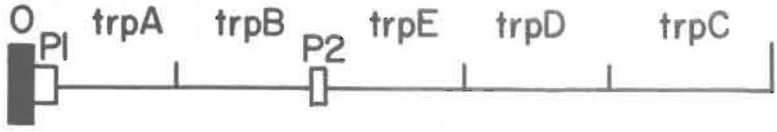
Mechanism of Gene Expression and Regulation

These genetic and biochemical studies of the tryptophan *(trp)* operon of *Salmonella typhimurium* are directed at two major and interrelated problems: (1) the mechanism of gene expression, especially its initiation, and (2) the nature and role of multifunctional enzyme complexes, including allosteric effects. In earlier studies we isolated mutant strains which show deletions of the operator terminus of the operon (Bauerle and Margolin, 1967). Since the initiating element of the operon is lost in these strains, expression of both deleted genes and remaining intact genes is prevented. Strains that have regained expression of these inactive, yet intact, genes have now been selected. Apparently, one mechanism of reinitiation is the generation of new initiator sites, closely linked to the termini of the deletions, by the action of mutagens that induce single base-pair transitions (Margolin and Bauerle, 1966). Successful mutagenesis has been accomplished with 2-aminopurine, nitrosoguanidine and diethyl sulfate. A collection of such strains has been made, and in all cases the mutagen-induced initiator site is separable from the parental deletion by recombination. Studies have been made of two independent initiator mutations, which were induced in a deletion strain that terminated *in trpA*, the first gene of the operon. Both are able to act as initiators in other operator-deletion strains. In both these strains, carrying only the new initiator mutation, the product of the *trpA* gene (one sub-unit of the anthranilate-synthetase complex) has greatly altered properties: the enzyme shows only about 1% of wild-type activity, it exhibits a K_m 60-fold higher than the wild-type enzyme, and it is hypersensitive to feedback inhibition by tryptophan and its analogues. Attempts are underway to purify the mutant proteins in order to study the change(s) induced in primary structure. Investigation of these artificial initiator elements might clarify the identity of the naturally occurring elements.

In pursuit of the relationship of enzyme complexes and allosteric control, we are studying the properties of anthranilate synthetase, the first enzyme specific to the tryptophan biosynthetic pathway. The enzyme is a complex of two dissimilar subunits (Bauerle and Margolin, 1966). Interestingly, one subunit possesses an additional activity (the second step of tryptophan biosynthesis). The complex is also the site of the allosteric control of the pathway by the end product, tryptophan. Purification on an analytical scale yields an essentially homogeneous enzyme. Preparative-scale experiments which are now in progress should help to elucidate the nature of sub-unit structure and allosteric interaction.

Bauerle, R. and P. Margolin, 1967. J. Mol. Biol. *26*, 423
Margolin, P. and R. Bauerle, 1966. Cold Spg. Harb. Symp. Quant. Biol. *31*, 311
Bauerle, R. and P. Margolin, 1966. Cold Spg. Harb. Symp. Quant. Biol. *31*, 203

Ronald H. Bauerle



Tryptophan operon in Salmonella

H. BREMER, HILDEGARD MICHALKE, K. MUELLER

Messenger RNA Synthesis In vivo and In vitro.

Rate of messenger RNA (mRNA) synthesis in E. coli. In a study of the regulation of RNA synthesis, it is desirable to establish a method of measuring the rate of synthesis. Since mRNA is unstable and is only a minor fraction of total cellular RNA, its amount in the cell and its synthesis rate had not so far been directly determined. Estimates based on indirect evidence suggested that about 2% of *E. coli* RNA is mRNA, that this mRNA has a lifetime of a few minutes, and that it is synthesized at a rate several times that of stable (mostly ribosomal) RNA. After growing *E. coli* with radioactive uracil, we determined the rate of radioactive UMP incorporation into RNA and also the kinetics of the specific radioactivity changes in the cellular pool of UTP. The quotient "incorporation rate/specific radioactivity", extrapolated to zero labelling time (when labelled mRNA has not yet decayed), corresponds to the total rate of RNA synthesis (stable rRNA and tRNA, and unstable mRNA). Subtracting the synthesis rate of stable RNA (= the same quotient obtained after long labelling time), the rate of mRNA synthesis was found to be about 2000 molecules (of average length 1500 nucleotides) per minute per cell. This would permit every gene on the *E. coli* chromosome to be transcribed about once every minute.

RNA chain-growth rate in vivo. By analyzing the sedimentation distributions of radioactive mRNA from T4-phage-infected *E. coli,* labelled for short times with radioactive uridine, the mRNA chain-growth rate was estimated to be 28 nucleotides/sec at 37°C. This means that the synthesis of a long polycistronic mRNA molecule may last several minutes. Knowing this rate of chain growth and the total rate of RNA synthesis (see preceding), the number of functioning RNA polymerase molecules in an *E. coli* cell producing messenger RNA can be estimated to be about 2000.

RNA chain-growth rate in vitro. The *in vitro* RNA chain-growth rate has been previously determined to be 2.5 nucleotides/sec (Bremer and Konrad, 1964; Bremer *et al,* 1965; Bremer, 1967). By analyzing the incorporation of ratioactive adenosine into 3′ termini (the growing ends) of nascent RNA molecules after very short labelling times, it is now found that *E. coli* RNA polymerase, during its operation on T4-DNA templates, makes temporary stops lasting several seconds; between the stops, RNA molecules grow quite fast, approaching the *in vivo* rate (see preceding).

RNA synthesis in UV-irradiated E. coli. To aid future studies of *in vivo* mRNA synthesis on viral DNA templates that do not stop bacterial RNA synthesis (e.g., phage T1), it appeared desirable to study the suppression of bacterial RNA synthesis by UV radiation. After UV irradiation of *E. coli B,* as well as of radiation resistant (B/r) and hypersensitive (B_{s-1}) strains, we measured the synthesis rate and sedimentation velocity of the radioactively labelled RNA. All strains gave the same result: immediately after irradiation the "number of RNA molecules initiated/min" is somewhat less than before irradiation, and with increasing doses RNA molecules become increasingly shorter. From the reduction in the RNA chain length, it is estimated that every UV hit that would inactivate colony formation in the most sensitive strain stops transcription at that site in all strains. Indirect evidence suggests that, after being stopped at a UV hit, both the RNA polymerase and the unfinished RNA molecule are immediately liberated from the template.

Bremer, H., 1967. Molec. Gen. Genet. *99,* 362
Bremer, H. and M.W. Konrad, 1964. Proc. Natl. Acad. Sci. *51,* 801
Bremer, H., M.W. Konrad, Kathleen Gains, and G.S. Stent, 1965. J. Mol. Biol. *13,* 540

C. BRESCH

Studies of Meiosis in Yeast

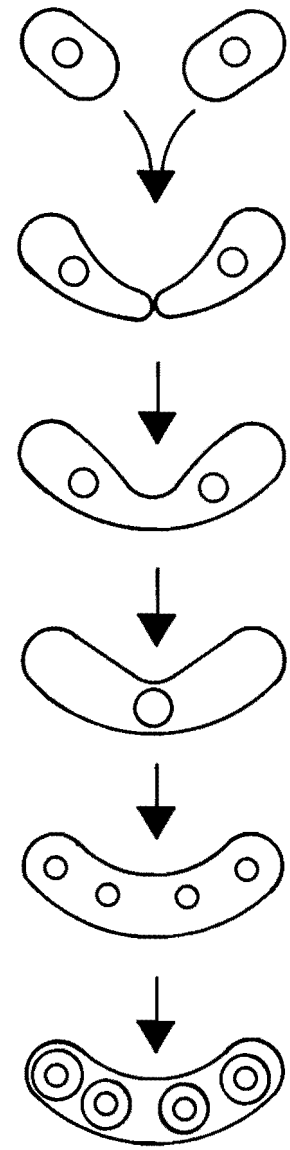
The phenomenon of meiosis, though fundamental in biology, is as yet barely understood beyond the descriptive level. In an attempt to identify special genes essential to meiosis, a search for mutants blocked at different stages of meiosis was undertaken. For these studies, the organism *Schizosaccharomyces pombe* was chosen. This fission yeast can be handled very much like bacteria and is one of the lowest forms of life showing meiosis. Its cytological limitations (no chromosomes can be differentiated by light microscopy) will hopefully be compensated by the simplicity of the organism. The main reason for the choice was the possibliity to select mutants blocked in meiosis by the following procedure:

Within a colony on an agar plate, pairs of cells of a self-fertile strain can undergo the consecutive events of conjugation, meiosis, and sporulation. A colony of a mutant cell, defective in either the ability to conjugate, to undergo meiosis, or to sporulate, will thus show no spores. Sporulation in colonies can be macroscopically detected by the dark blue stain which develops after treatment of the plates with iodine vapor. Colonies without spores remain yellowish.

By this technique, hundreds of spontaneous and UV-induced mutants have been isolated, most of which are found to be blocked at the first step (i.e., conjugation). To identify those blocked at a later stage, a second screening was used. Non-sporulating colonies were examined microscopically and mutants showing cell fusion were selected. The separation of mutants defective in meiosis from those defective in sporulation was similarly achieved. Staining with acridine dyes permits preliminary identification of normal meiosis leading to 4 nuclei. The majority of mutants showing cell fusion were of this type and therefore apparently blocked in sporulation. However, mutants with blocks at different stages in meiosis could also be distinguished.

The large number of mutants obtained were submitted to a complementation test, i.e., crosses were performed between pairs of independent mutants and examined for ability to sporulate. Successful sporulation indicated mutations blocked at different functions, inability to sporulate suggests blocks in the same function. No mutation has as yet been observed which could be classed as dominant in preventing sporulation in combination with all other partners.

So far, 8 complementation groups (genes) have been identified blocked in meiosis, while 18 others are apparently blocked in sporulation. These numbers may exhaust the possibilities of the selection technique, since 200 additional isolations did not increase the number of mutant groups.



Stages in yeast meiosis

Hermann Bujard

H. BUJARD

Structural Studies of Large Molecules

The actinomycin-DNA complex has been investigated by electron microscopy, in collaboration with Dr. D. Lang. Reversible intra- and inter-molecular crosslinks have been demonstrated. Contrary to conclusions drawn from a variety of other physical and chemical data, no evidence has been found that actinomycin intercalates into the Watson-Crick helix.

A procedure was developed to isolate bovine papilloma virus, a representative of the papova group of tumor viruses. The DNA of this virus has been characterized by sedimentation studies and electron microscopy. The molecular weight is 4.9 x 10^6 dal. The molecule is circular and possesses tertiary turns. Sedimentation studies show that the sense of the turns is right-handed, and the number of turns in one molecule is 18 ±3. Together with the data known from the similar but smaller DNA of polyoma virus, it would appear that, under comparable conditions, the number of tertiary turns in circular twisted DNA is proportional to molecular weight. Its unique structural configuration makes this type of DNA an interesting object for a number of physical and biochemical studies.

Bujard, H. 1967, J. Virol. *1*, (in press)
Bujard, H. 1968, J. Mol. Biol. (in press)

R.C. CLOWES, ADELAIDE MACFARREN, CHRISTINE SMITH, T. NISIOKA

Extrachromosomal Factors in Bacteria

This program continues an investigation of a series of colicin (Col) factors, and certain infectious, multiple-drug-resistance (R) factors, together with the F fertility factor. The work was done primarily in *Escherichia coli* K12, in which all these act as transfer or sex factors.

Repression of F. In stably infected cells, we have found that certain ColB factors (ColB1, ColB2, ColB5) give rise to a self-repressed fertility system as do certain R factors (Rfi^+ 222). The factors are derepressed in newly infected cells, as shown by the production of cell receptors, which take the form of F-like pili to which male-specific phages (μ2, f2, MS2) adsorb. In a cell possessing the F sex factor and one of these factores, the repression extends to F and the cell does not usually show fertility.

Another factor (ColB3), however, showing similar self-repression, does not repress F. It is found in the wild state associated with an F-like element, which resembles closely the standard F factor but is not co-immune with it. These two F factors are capable of stable co-existence.

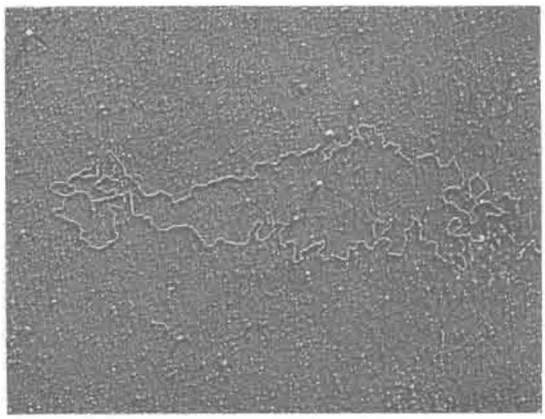
Curing. Experiments on "curing" or elimination of factors from cells have been extended by the use of ICR (nitrogen half-mustard) compounds. These compounds "cure" all extra-chromosomal factors so far investigated, even those refractory to acridine-orange (AO) curing (ColI, Rfi^-), as well as all other factors when they are present in recombinaseless *(rec^-)* strains. AO also appears to cause mutation, provided the F sex factor is present (Clowes and Moody, 1967).

ColV factors. Genetic experiments with *ColV* factors have been continued and extended (MacFarren and Clowes, 1967).

Electron micrograph of DNA from Bovine papilloma *virus, supertwisted and relaxed forms, x60,000 (taken by Dimitrij Lang)*

The ability of the factor ColV2 to integrate with the chromosome is evidenced by the isolation, from strains infected with ColV2, of strains with enhanced and oriented chromosomal transfer. Further investigations are aimed at clarifying details of the mechanism of recombination between sex factors and chromosome.

Physical studies. Experiments intended to investigate the physical nature of Col factors, as well as pair-wise interaction of factors, have been delayed by the finding that Col factors transferred to strains of *Proteus mirabilis* are unstable. No direct method of selective pressure, as in the case of R and F factors with integrated selective (e.g., lac^+) chromosomal markers, is possible. Certain indirect selective methods are being developed. Meanwhile, in collaboration with Drs. Lang and Bujard, other selectable factors are being used in an effort to develop the techniques and possibly to extend them, using ethydium bromide treatment and electron microscopic examination. Evidence has now been obtained of the circularity of the R factors R222 and R15, and the length of these structures has been found to be 28 and 18 μ respectively, corresponding to molecular weights of 56 and 36 x 10^6 daltons.



Electron micrograph of DNA from infectious drug resistance factor (R15), x16,000 (taken by Michiko Mitani)

R.C. Clowes and E.M. Moody, 1967. Microb. Genet. Bull. *26,* 4.
MacFarren, A.C. and R.C. Clowes, 1967. J. Bacteriol. *94*, 365.

M. COVAL

Structure and Activity of Enzymes

In the presence of peroxide, the enzyme thyroid peroxidase catalyzes the iodination of tyrosine residues of proteins. It is therefore of importance to the understanding of thyroid physiology. The properties of this enzyme and, in particular, the presence of a heme group at the active site, are in dispute, largely due to the crude preparations so far available. In collaboration with Dr. Alvin Taurog of the University of Texas Southwestern Medical School, this enzyme has been extracted from hog thyroids and a thousand-fold purification obtained. The physical-chemical and enzymic properties of the peroxidase have been studied.

A phosphatase from frog ovaries, specific for high-energy phosphate monoesters, has been purified several hundred-fold. Its molecular weight is 56,000. It shows only one sedimenting boundary (Martinek and Coval, 1966), but electrophoresis shows two or three remaining protein bands in addition to the active band. The enzyme is activated by reducing agents and inactivated by oxidizing agents, both reversibly. Kinetic measurements of K_m vary over a fifty-fold range (7 x 10^{-5} to 3.3 x 10^{-3}M) and the V max values over a two-fold range (4.9 to 9.7) for a number of different substrates.

An acid phosphatase has been partially purified from beef spleen. The isolation is continuing and kinetic properties are being determined. This enzyme appears to be analogous to the frog egg enzyme in substrate specificity, pH optima, activation, and inhibition.

An investigation of mouse teratomas, which spontaneously develop in the testes of mice, has been initiated. Grown subcutaneously, these tumors produce large organized and differentiated structures resembling various adult tissues, whereas when grown intraperitoneally as ascitic tumors, they resemble five- or six-day-old mouse embryos. We find that the levels and isozyme patterns of lactic dehydrogenase and acid phosphatase, both in the embryoid bodies and in the original non-tumorous testicular tissue, are related. Three different teratomas, a pleomorphic, a myoblastic, and a neural form, are being main-

tained as solid tumors. A known ascitic form of the pleomorphic tumor is also being maintained and the ascitic forms of the two other tumors have been derived. The strain specificity of the solid and ascitic forms of these tumors appears to be different. These tumors are of interest because they lend themselves to studies of development, regulation, tumor metabolism, and perhaps the early mammalian embryo.

J. Martinek and M.L. Coval, 1966. Fed. Proc. *25*, 342.
Taurog, A. and M.L. Coval, VIIth Int. Cong. of Biochem. 1967.
Coval, M.L. and A. Taurog, 1967. J. Biol. Chem. *242*, 23.
Coval, M.L. and A. Taurog, 1967. Fed. Proc. *26*, 643.

Yeast laboratory

H. GUTZ, P. ANGEHRN, H.J. TREICHLER

Molecular Genetics of Yeast

The main focus of this work has been on gene conversion and meiosis in the yeast *Schizosaccharomyces pombe.* In addition, experiments were continued to map genes of hitherto unknown locations by haploidization.

Studies on gene conversion. One *ade-6* mutant (M26) of *S. pombe* is remarkable for giving high conversion frequencies. The conversion event was studied in 10 different crosses by tetrad analysis, 6503 asci being analyzed using micromanipulation techniques. In crosses with other *ade-6* mutants, it was found that *M26* is frequently converted to the other allele even when the map distance between the two sites is about half the total *ade-6* map. Since gene conversion appears to involve a repair process in hybrid DNA, these results would appear to be a direct proof that a single conversion event involves large regions of hybrid DNA extending between the two sites of mutation. Furthermore, the results indicate that hybrid DNA within the *ade-6* locus is more frequently formed in the presence of *M26* than in crosses without *M26.* This unusual property cannot easily be explained by current hypotheses on recombination.

"Twin meiosis". In experiments with diploid strains of *S. pombe* a rather unexpected phenomenon has been found (Gutz, 1967 a & b). When diploid strains of compatible mating type are crossed, in addition to the expected asci with 4 diploid spores, asci with 8 haploid spores are formed. Using strains with suitable markers, it was shown that, in the latter case, after cell fusion, karyogamy does not occur but the diploid nuclei undergo separate meioses (twin meiosis). Even in crosses between diploid strains of opposite heterothallic mating types (such strains being incapable of sporulation), twin meiosis occurs in the fused cells. Obviously both the diploid nuclei are able to complement each other for meiosis.

In crosses between haploid and diploid strains, a few of the asci have 6 haploid spores. Four spores possess the markers of the diploid parent, and the other two possess the markers of the haploid parent. The 6-spored asci apparently originate from zygotes in which, after plasmogamy, karyogamy does not occur, but the diploid nucleus undergoes meiosis whereas the haploid nucleus undergoes mitosis (Gutz, 1967b).

Haploidization experiments. It was found earlier that diploid strains of *S. pombe* can be haploidized by treatment with p-fluorophenylalanine (Gutz, 1966). These experiments have been continued to map genes at hitherto unknown locations. So far, eight more genes have been located in linkage groups I and II, respectively.

Gutz, H., 1967a. Science *158,* 796
Gutz, H., 1967b, Ber. Deut. Bot. Ges. *80*
Gutz, H., 1966. J. Bacteriol. *92,* 1567

K. HAEFNER

The Expression of Killing and Mutation in Irradiated Yeast and E. coli.

This work is focused on improving our understanding of radiation effects on cells. The program is concerned with (1) investigating the causes for radiation-induced lethal sectoring and (2) studying mutation segregation after ultraviolet irradiation.

Lethal sectoring (LS). (a) Ultraviolet (UV) and X-ray-induced LS have been studied by means of micromanipulation in yeast and *E. coli* strains of different radiation sensitivity. In general, a positive correlation has been found between UV-induced LS and radiation sensitivity (Haefner and Striebeck, 1967). However, this correlation does not apply to X-ray-induced LS in haploid *Saccharomyces.* Highly sensitive haploid *Saccharomyces* strains show no LS after X-irradiation. From the results it is reasonable to assume that LS is related to intracellular repair processes going on after the first post-irradiation cell division. (b) X-ray-induced LS was extensively studied in wild-type haploid and diploid *Saccharomyces* for doses smaller than 12 Krad. In this dose range, where up to 96% inactivation is observed in the haploid strain, no LS has been observed (Haefner, 1967a). (c) Several strains of yeast and *E. coli* have been found which show a constant rate of spontaneous lethal sectoring. They are under further investigation as a model for radiation-induced LS.

Mutation segregation. The frequencies of UV induction of pure and mixed mutant clones have been studied, using individually irradiated cells and micromanipulation techniques. Several cells from pure mutant clones of *Schizosaccharomyces pombe* have been investigated genetically and found always to contain the same homo-allelic mutation. The relevance of these findings has been discussed in terms of mutation induction processes (Haefner, 1967b, c, and d).

Haefner, K. and U. Striebeck, 1967. Mutation Res. *4,* 399.
Haefner, K. 1967a. Rad. Res. *31,* 400
Haefner, K. 1967b. (Abstract) Rad. Res. *31,* XV. Ann. Meeting Rad. Res. Soc., No. Eg6.
Haefner, K. 1967c. Genetics *57,* 169.
Haefner, K. 1967d. Mutation Res. *4,* 514.

Walter Harm

W. HARM

Dark Recovery from Ultraviolet Lethal Damage

The distinction between two dark-repair systems in E. coli. Recently it became evident that dark repair controlled by the *hcr* (or *uvr*) genes (*excision-resynthesis repair,* or ERR) is distinct from dark repair controlled by *rec* genes *(REC repair).* Comparison of the properties of 4 closely related strains, active in both, only one, or none of the dark repair systems, has led to the following results: (a) Infecting UV-irradiated phage T1 is repaired by the ERR system only, wheras phage λ shows in addition some repair by the REC system. (b) Liquid-holding recovery is an expression of enhanced ERR repair. It can occur only in strains with an active ERR system regardless of REC properties. (c) Both dark-repair systems are inhibitable to some extent by either acriflavine or caffeine. The UV survival of a strain lacking both dark-repair systems is not influenced by these substances.

The effect of dose fractionation and dose protraction on the dark repair of UV lethal lesions in E. coli. It was found that the ultraviolet survival of wild-type *E. coli* strains (such as B/r, C, B) can be raised appreciably by applying the UV dose in two or more exposures and allowing time for ERR repair between. An extremely high survival was found with either highly fractionated irradiation or continuous irradiation at a very low dose rate (80 ergs/mm^2/hour) for several days. Under these conditions the survival curve of B/r was quite exponential, lacking the "shoulder" usually found. The high survival under these conditions reflects more extensive ERR dark repair than usual. Several lines of evidence suggest that at high UV doses a steady state is reached in which the lesions are repaired as fast as they are produced. The lower extent of repair under normal conditions apparently results from accumulation of too many UV lesions, which interfere with each other in repair.

Dark repair of all of the photo-repairable UV lesions in E. coli. The survival of *E. coli* B/r, UV-irradiated at a dose rate of 8 ergs/mm^2/sec, increases from $\approx 10^{-4}$ to $> 10^{-1}$ as a result of maximum photoreactivation (PR). Holding the irradiated cells in buffer prior to allowing PR, progressively decreases the maximum PR survival that is observed, but increases the dark survival *(liquid-holding recovery).* Both of these changes are due to excision of dimers; they are essentially absent in the non-excising strains B_{s-1}, AB2437 and AB2480. The decrease in maximum PR survival of B/r indicates that potentially photo-repairable lesions are excised, but dark repair is never completed. While some PR is still observed after holding the irradiated cells for 24 hours, PR is fully absent in B/r cells irradiated for 24 hours at very low UV dose rate (0.022 ergs/mm^2/sec), a condition allowing most extensive dark repair. Comparative experiments with *rec*$^-$ and wild-type strains indicate that both of the basic dark-repair systems (ERR and REC repair) must function in order to have a total disappearance of observable PR.

A "negative" liquid-holding effect. UV-irradiated *E. coli* C shows a decreased survival when it is held in buffer prior to plating, whereas most *E. coli* strains show increased survival (liquid-holding recovery) under these conditions. Studies with repair inhibitors and at varying temperatures, as well as photoreactivation studies, indicate that the "negative" holding effect in *E. coli* C involves the same lesions that are operated upon by liquid-holding recovery and which are dark repairable by the ERR mechanism. Whether an increase or a decrease in survival results from holding in buffer depends on the genotype of the organism and (to some extent) on the particular experimental conditions.

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D. HARRIS

Active Site of a Hydrolytic Enzyme

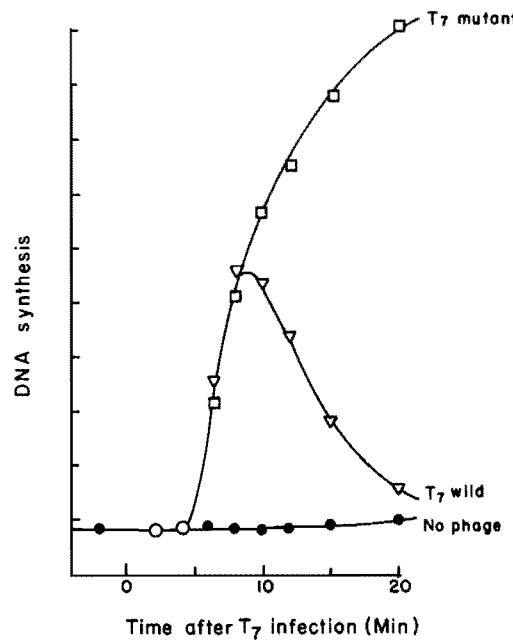
In a continuation of the investigation of a phosphoanhydride hydrolase we have studied the effect of pH on the kinetic constants (K_m and V_{max}) of the enzyme reaction. On the acid side of the pH optimum, V_{max} is constant but K_m increases with increasing pH, suggesting the presence of a group in the enzyme with a pK of approximately 2.5 which is important for binding the phosphate anion. On the alkaline side, the affinity of the enzyme for substrate is independent of pH (K_m is constant) but V_{max} decreases with increasing pH. The results can be plausibly explained by postulating a positively-charged group within the enzyme having a pK of approximately 4.5 which exerts an electrophilic withdrawal on the bridge oxygen. Attempts to identify this group within the enzyme are being undertaken.

R. HAUSMANN, BEATRIZ GOMEZ

Mutual Exclusion between Phages T3 and T7

Bacteriophages serologically related to T3 show different degrees of genetic and physiological incompatibility when crossed with each other. The pair T3 and T7 was chosen as a model system for studying these effects. Only a fraction (1 to 20%) of the host cells yield both phage types when simultaneously infected with T3 and T7. The other cells produce either one or the other phage. Conditionally lethal mutants (amber and temperature-sensitive mutants) of these phages have previously been isolated and studied. Recently, special attention has been given to those amber mutants unable to initiate phage-directed DNA synthesis ("early" mutants). Although several complementation groups of "early" mutants have been individualized by spot tests, burst-size measurements in non-permissive cells established only two clear-cut units of functional complementation, both in T3 and T7. Further studies of these two groups (Hausmann and Gomez, 1967) have concerned patterns of phage-directed uridine incorporation, ability to break down host DNA, ability to exclude the heterologous wild-type phage, and ability to complement heterologous amber mutants. It was found that mutants of one group, termed DO-A, unlike the wild type and the mutants of the other group (DO-B), were unable (1) to promote an efficient phage-directed uridine uptake, (2) to promote efficient breakdown of host DNA, and (3) to exclude the heterologous wild type. Representatives of both groups, DO-A and DO-B, unlike representatives of some other complementation groups, were totally incapable of complementing any function in the hyterologous wild types. The data suggest that mutual exclusion is a relatively early event, although synthesis of one "early" enzyme of T3 seems not to be affected by it (Hausmann, 1967).

We have also given attention to physiological and genetic aspects of abortive infection of *Shigella sonnei* (strain D_2 371-48) by bacteriophage T7. Phage T7 is unique, as far as we could test it, in its capacity to lyse this shigella strain without subsequent phage production. We have shown that T7 initiates phage-directed DNA synthesis in this host, but the pool of phage-directed DNA is rapidly destroyed just before one would expect the first mature progeny particles. Mutants of T7 growing normally on this host have been isolated. However, upon mixed infection with T7 wild type, the abortive phenotype is dominant. Mutants phenotypically similar to T7 wild type were isolated from the normally growing mutants. These revertant mutants all map at one site, adjacent to the right end of the DO-B complementation group of T7. Now under investigation is whether this self-exclusion of T7 can be correlated with mutual exclusion between T3 and T7.



Abortive infection of Shigella *by T7 phage*

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K. HECKMANN

Genetic Studies in Ciliates

Age-dependent intraclonal conjugation in Euplotes crassus. This work has recently been completed (Heckmann, 1967), and shows the following results: (a) Intraclonal conjugation in *E. crassus* is a consequence of a breakdown of the dominance relationship between mating-type alleles, allowing previously recessive alleles to come to expression in some of the cells. (b) This breakdown of dominance takes place regularly at a clonal age of about 500 consecutive fissions, and seems to serve a biological function similar to that of autogamy in other ciliates. (c) The change from the life cycle stage of mating-type stability to the life cycle stage of mating-type instability is under the control of at least one other gene unlinked to the mating-type locus.

Killer trait in Euplotes minuta. In the previous progress report it was mentioned that we had found bacteria-like particles associated with the killer trait of certain stocks of *Euplotes minuta.* A description of these particles and a characterization of the killer activity, suggesting that the cytoplasmic particles are themselves the bearers of the toxic activity, is now published (Heckmann, Preer and Straetling, 1967). It was also noted that stock VF17 showed a different way of killing the indicator *E. crassus* strain D, although differences between the particles of stock VF17 and those of the stocks K1, K3 and K7 could not be found. Experiments cross-breeding stock VF17 with sensitive stocks now reveal that VF17 is a mate-killer. Sensitives are killed as a consequence of conjugation with this killer, whereas all the other killers show no effect on sensitive cells during conjugation and can therefore be rescued by re-isolation after separation from their "killer-mate".

Nuclear control of morphogenesis in ciliates. The cortex of ciliates exhibits a high degree of structural differentiation. This and the occurrence of sexual and asexual reproduction, together with the ease of culture and experimental manipulation, makes ciliates pre-eminently suitable for studying those factors responsible for the production and maintenance of cellular structure. Recent studies of "cortical" heredity in *Paramecium and Tetrahymena* indicate that the arrangement of surface organelles is dictated by pre-existing orientations, and that differentiation of particular structures may be induced by material emanating from pre-existing structures of the same type (Beisson and Sonneborn, 1965; Nanney, 1966). However, the molecular basis of these orientations and inductions is obscure, and the continuity of such inducing systems is open to doubt. As pointed out by Beale (1966), surface heredity may be a property aiding maintenance and differentiation of surface structures at cell division but ultimate long-range control might still come from elsewhere, presumably the nucleus.

Klaus Heckmann

A possibility for testing this idea arose recently. It was found that two interfertile stocks of *Euplotes minuta* differ with respect to the expressed number of kineties (Heckmann and Frankel, unpublished). Cross-breedings between cells of these two stocks led to progeny which, after 40 to 60 consecutive fissions, expressed the number of kineties characteristic of *one* of the parental stocks, regardless of cytoplasmic origin of the progeny lines. In an F2 obtained by autogamy, segregation into the parental cortex types was found. These preliminary results indicate that in fact the cortical differences between these two stocks are subject to a long-range control by nuclear genes and open the possibility for studying the interplay of genic and non-genic factors in controlling the formation of new surface structures.

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W. HEUMANN

Conjugation in Star-forming Rhizobium

It has been shown that star formation in bacteria is a process of conjugation involving chromosome exchange followed by recombination at the star-center. This process differs in many important respects from *E. coli* conjugation, particularly in showing no sexual differentiation between mating cells.

Most genetic experiments have so far used a *Rhizobium lupini* strain, isolated some years ago from root nodules of *Lupinus luteus*. In order to test cross fertility between rhizobium strains of different origin, some Hungarian *R. lupini* strains, kindly donated by Dr. Magda Gabor of the Hungarian Academy of Science, have been recently used. A series of different auxotrophic mutants were isolated from these strains by treatment with nitrous acid. Some mutants were fertile with our rhizobium mutants and confirmed previous linkage relationships. Some mutants produce a gum that prevents fertility: only those mutants losing gum production were cross fertile with each other and with our *R. lupini* mutants.

We are also studying the differential effect of sunlight on star-forming strains and their non-star-forming counterparts. Results obtained so far indicate that star formation, or conjugation during star formation, repairs radiation damage: radiation doses lethal to non-star-forming mutants were survived by star-forming strains.

Czygan, F.C. and W. Heumann, 1967. Arch. Mikrobiol. *57*, 123.

Wolfram Heumann (left) receives the State of Bavaria's official appointment as Chairman, Department of Microbiology, University of Erlangen. Making the presentation is Gershon Canaan, Acting German Counsul in Dallas

H. HIROKAWA, I.C. FELKNER

Transformation in Bacillus subtilis

Recombination studies on the DNA molecule. Current work is concerned with the genetic configuration of recombinant DNA formed between two donor DNA s in protoplasts, using clonal analysis of transformed cells.

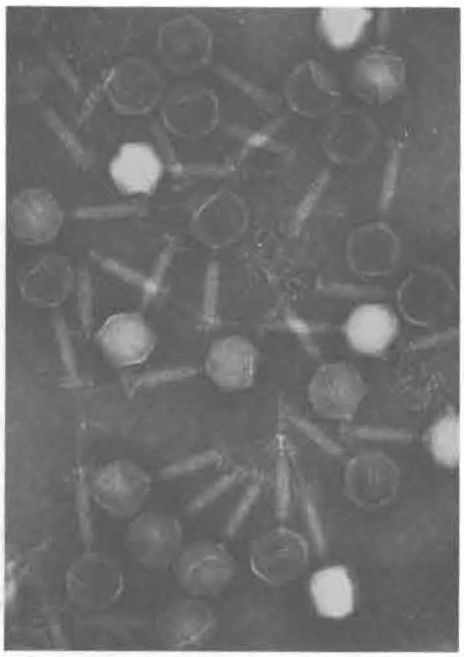
Two types of hetero-duplex DNA with single linked *ind*$^-$ or *his*$^-$ markers (e.g., $\frac{h^+ \quad i^+}{+ \quad -}$ or $\frac{h^+ \quad i^+}{- \quad +}$) are made by heating and slow cooling of the appropriate DNA mixtures at different concentrations. Recipients requiring histidine plus indole are treated with either of the hetero-duplex DNAs and then plated on minimal medium supplemented with either L-histidine or L-tryptophan. Clonal analysis for unselected markers in transformed cells reveal a disproportionately high fraction of mixed clones. This leads to the hypothesis that in *Bacillus subtilis* transformation, both strands of donor DNA may integrate with the recipient chromosome. This hypothesis does not necessarily contradict the conclusion from biochemical analysis (Bodmer and Ganesan, 1964) that single strands integrate.

To determine whether recombinant DNA formed in protoplasts between two donor DNAs is hetero-duplex, a similar clonal analysis was undertaken using wild-type DNA (a homoduplex) as a control. The number of mixed clones formed by recombinant DNA or by wild-type DNA was negligible. This suggests that the genetic composition of recombinant DNA is a homoduplex, like wild-type DNA. It also suggests that recombination between donor DNAs in protoplasts may take place at the level of duplex DNA.

Previous biological experiments indicated that DNA uptake by protoplasts was higher than by competent rod cells (Hirokawa and Ikeda, 1966). DNA uptake by protoplasts is now being studied, using biochemical and biophysical methods. Recent results show that tritium-labelled DNA is incorporated into protoplasts and that the rate of DNA uptake by protoplasts is significantly higher than in either competent or non-competent cells.

Competence studies. Although induction of "competence" in *B. subtilis* (Felkner and Wyss, 1965; Bott and Wilson, 1967) has been studied, and a factor associated with competence has been isolated, (Felkner and Wyss, 1964; Charpak and Dedonder, 1965; Akrigg *et al.*, 1967) the nature of this phenomenon, which controls DNA uptake, is not understood. The most serious deterrent to defining competence in quantitative terms has been the lack of an assay which clearly separates it from subsequent processes (e.g., recombination) leading to a genetically transformed cell.

An effort was made to construct mutant strains which could be used for specific assay of competence. The properties sought were requirement for thymine *(thy⁻)* and the inability to carry out molecular recombination *(rec⁻)* while retaining the capacity for DNA uptake. A new technique (Felkner *et al.*, 1967) permitted the isolation of thymineless strains. Some were derived from an ultraviolet-sensitive strain, because of the possible connection between the processes of recombination and repair of UV damage. Recombination studies with these mutants show that they are at three distinct sites. The inter-mutant recombination frequency is a hundred-fold greater than between pairs of mutants within the same gene, and approaches 10% (the frequency with wild-type DNA) from which it is inferred that there are two, and possibly three, distinct *thy⁻* loci. Highly labelled tritiated-thymidine DNA can be isolated from these strains. A mutant has been isolated from one *thy⁻* strain which takes up DNA at least as well as its parent but does not produce recombinants. One of the *thy⁻* mutants gives rise to *rec⁻* strains at a very high frequency and suggests possible phenotype interaction between these two loci.



Electron micrograph of B. subtilis *phage SP50, x90,000 (taken by Dimitrij Lang)*

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J. JAGGER, H. TAKEBE

Ultraviolet Damage and Protoreactivation in Bacteria

Induction of division delay by near-ultraviolet radiation. The action spectrum for induction of division delay in *E. coli* B by near-ultraviolet radiation, as measured with an electronic cell counter, has been found to be similar to the action spectra for growth delay and for photoprotection (Phillips, Person, and Jagger, 1967). These and related findings indicate that all three effects are induced by a common type of critical event, and that this event is a photochemical change occurring outside the genome.

Induction of growth delay by far-ultraviolet radiation. Logarithmic-phase cells of *E. coli* B/r were irradiated with rapid stirring at about 10^8 cells/ml by different wavelengths of UV from 230 to 295 nm. The doses were low enough to permit more than 20% survival. Growth delay after dilution was measured by optical density, using a Bonet-Maury Biospectrophotometer. Corrections were made for (1) apparent delay caused by killing and (2) absorption of the cell suspension. The action spectrum of growth delay thus obtained is similar to the absorption spectrum of nucleic acid and is not similar to the absorption spectra of isoprenoid quinones. Therefore, although absorption of UV by quinones above 300 nm may well be responsible for the induction of growth delay, such absorption does not appear to play a significant role in the induction of growth delay below 300 nm.

Photoreactivation in Streptomyces griseus. An action spectrum for photoreactivation (PR) in conidia of this organism has been obtained, using narrow bands of monochromatic light (Jagger, 1967). The spectrum confirms the peak originally found by Kelner (1951) at 440 nm, but does not show the suggestions of fine structure that he reported. We have extended the spectrum below 366 nm into a region not studied by Kelner, and have found a new peak at 313 nm. The rate of PR at 440 nm is dependent upon temperature during PR treatment and upon the dose rate of the photoreactivating light, from which we conclude that PR at this wavelength is due to activity of the PR enzyme. At 313 nm, however, there is only slight dependence upon temperature and dose rate, suggesting that PR at this wavelength does not utilize the PR enzyme. The absence of photoprotection and liquid-holding recovery in this organism indicates that this PR is not similar in mechanism to photoprotection and is therefore not like the "indirect PR" found in *E. coli.* It therefore appears to be a third and previously unknown type of PR.

A mutant *(phr⁻)* was isolated which has a very low efficiency of PR, and which shows PR only at wavelengths 313-365 nm, with a maximum at 313 nm. PR in the mutant shows no temperature or dose-rate dependence, and we conclude that the mutant lacks the PR enzyme. Several UV-sensitive mutants have also been isolated (Takebe and Jagger, 1967), including one that was isolated from the *phr⁻* mutant. This one also showed a small PR at short wavelengths, and presumably has poor dark-repair ability. These mutation studies suggest that PR in *S. griseus* at 313 nm (1) is not mediated by the PR enzyme, and (2) does not involve dark repair. They therefore support our hypothesis that this PR involves a third kind of mechanism. Our results are not inconsistent with the idea that this third mechanism could be a direct photochemical reversal of the 312-nm-absorbing UV photoproduct recently described by Varghese and Wang (1967).

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W. KRONE, H. BRUNSCHEDE

Biochemical Effects of Human Chromosomal Aberrations

UDP Gal-4-epimerase. Attempts to compare enzyme activities of normal diploid fibroblasts with those carrying chromosomal aberrations necessitated a thorough investigation of all the factors which influence enzyme activities of cultured cells.

Epimerase activity in particular had been shown to depend on the growth phase and on the age of the donor of the biopsy from which the cell cultures were derived (Krone and Brunschede, 1966a, b). Besides these, a number of factors were found to require control if meaningful comparative measurements are to be made. Both high pH of the medium and a low initial cell density decreased epimerase activity, the pH being more important. The antibiotics aureomycin and kanamycin, used to prevent infection of the cultures by mycoplasma, had highly variable effects on epimerase activity.

Winfrid Krone

Studies of the dependence of epimerase activity on the age of the donor of the fibroblasts, raised the following questions: (a) Is a different epimerase active in young and old donors? or (b) Is there one enzyme which decreases activity with increasing age? The latter interpretation seems to be correct: The Michaelis constants of the enzymes of both sources are very similar, if not identical, with the substrate UDP Gal as well as with the co-factor NAD. Both enzyme preparations behave identically in inhibition experiments with UDP-N-acetylglucosamine and the rates of heat inactivation under identical conditions are indistinguishable.

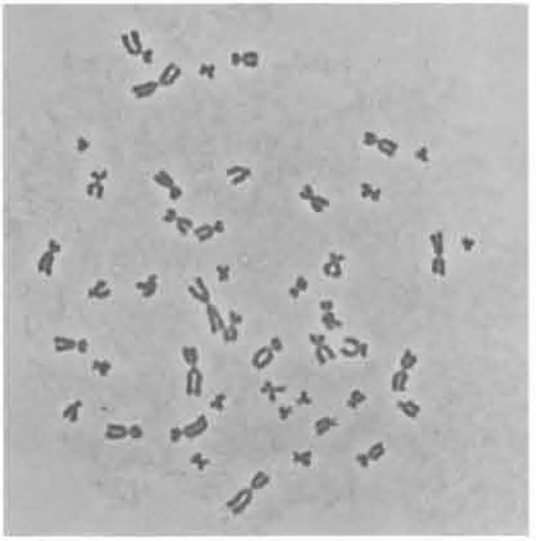
In order to develop additional means of comparing cells with various karyotypes, the studies with cells grown in galactose-containing medium were continued (Krone and Brunschede, 1967). With an improved medium, containing galactose instead of glucose, it was shown that epimerase activity is enhanced as much as 105% over its value in cells grown in the glucose-containing control medium. This effect is not due to the pyruvate which has to be added to galactose-containing media. It develops slowly during the lag phase, is most pronounced during exponential growth and decreases slightly during the stationary phase. Reversal of the medium (galactose → glucose, and vice versa) brings about the expected changes in epimerase activity. The stimulation of epimerase activity by galactose is sensitive to cycloheximide, an inhibitor of protein synthesis (Krone and Brunschede, 1967). When glucose and galactose are added to the extracts of cells grown on ordinary Eagle's medium, there is no difference in epimerase activity. Activation of the enzyme by galactose itself therefore seems unlikely.

Thioacetamide. The weak carcinogen thioacetamide (TA) inhibits the growth of human diploid fibroblasts *in vitro;* the concentration necessary to decrease the growth rate to 50% of the control value was found to be 75 μg/ml.

In the stationary phase, the cells seem to be rather resistant to thioacetamide. Treatment of stationary cultures with 250 μg TA/ml for 75 and for 240 hours did not affect the growth rate after recovery from TA and resuspension in fresh medium at low cell density. The RNA/DNA ratio, which increases rather drastically in liver cells *in vivo* after feeding of TA, was not significantly changed in stationary cultures of diploid human fibroblasts. These observations suggest that cell division may be a prerequisite for TA to exert its influence on fibroblasts *in vitro.*

For this reason, the influence of TA on the karyotype is currently being investigated. Cells were exposed to thioacetamide during their growth phase for various times and the karyotype studied after short-term growth in control medium. Although occasional abnormalities were observed (one dicentric chromosome and a fragment, besides some tetraploid cells) no consistent conclusion can yet be drawn from these studies. Therefore, a long-term experiment was started, in which the TA was added to the primary culture (stem culture), so that the outgrowth of the first cells took place in the presence of the drug. Preliminary observations show an increase in the percentage of aneuploid cells after the third passage, as compared to subcultures derived from the unexposed stem culture. Cells grown permanently under the influence of thioacetamide (75 μg/ml) show only about half as much growth inhibition by the same concentration of TA as control cells.

These studies should provide some insight into the mechanisms by which an oncogenic substance causes changes in the karyotype and into the relation of those alterations to concomitant biochemical events in the cells so affected.



Human chromosomes, x*1300*

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Krone, W. and H. Brunschede, 1967. Abstr., Symposium on Galactosernia, Chicago.

D. LANG

Quantitative Morphology of DNA

Diffusion-controlled adsorption of DNA onto a protein surface film (Lang *et al.,* 1967) made it possible for the first time to measure the *diffusion coefficient* of high-molecular-weight DNA (from T3- and χ-bacteriophage), by an electron microscopic method which permits counting of individual adsorbed molecules. The DNA concentration required (5 x 10^{-8} g/ml) is so low that such solutions behave ideally (in the physical-chemical sense) in spite of the great length of the DNA (12 and 20 μm). As a consequence, the diffusion coefficients measured are independent of concentration, in contrast to all other hydrodynamic methods. A theoretical analysis of the adsorption kinetics also showed that the adsorption of DNA onto a cytochrome c surface film is irreversible. Our method appears to have the potential of being a new approach in polymer chemistry.

The *effect of actinomycin and of intercalating substances on size and shape of DNA* has been directly shown by electron microscopy, in collaboration with H. Bujard. This investigation is not yet completed. However, it does appear that actinomycin condenses DNA by intramolecular cross-links, whereas an intercalating drug increases the length of DNA.

DNA from bacteriophage χ is presently under study. This linear DNA is found to expose one complementary single strand at each end and thus can form circles and dimers, behavior similar to that of the DNA from phage λ.

In cooperation with E.W. Gardner and T.O. McDonald of Texas Christian University, microtubules and particles resembling phage tails have been identified in a bacteriocinogenic strain of *Vibrio cholerae* (Lang *et al.,* 1968).

Electron micrography, metal shadowing

Lang, D., H. Bujard, B. Wolff and D. Russell, 1967. J. Mol. Biol. *23,* 163.

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YVONNE T. LANNI

The First-step-transfer DNA of Bacteriophage T5

One of the more interesting properties of bacteriophage T5 is its so far unique, mode of transfer of the viral DNA molecule into the host cell.

Formally, the T5 DNA molecule, and genome, can be divided into two parts: the "first-step transfer DNA" or "FST-DNA" and the rest of the molecule. After adsorption of the virus to the host cell, the viral FST-DNA is initially transferred to the host cell; there is a pause, followed by transfer of the rest of the DNA molecule if protein synthesis is allowed. After the FST-DNA is transferred, the rest of the DNA molecule can be removed by mechanical shearing, thus allowing the study of cells infected with FST-DNA only.

The FST-DNA has been purified and found to be double-stranded and to represent about 8% of the total molecule (McCorquodale and Lanni, 1964; Lanni *et al.*, 1964). Cells infected with FST-DNA only are killed as colony-formers, and are incapable, as expected, of phage production, but capable of supporting the growth of a superinfecting phage. When cells infected with FST-DNA only are allowed to synthesize proteins, one observes degradation of host DNA, as in normal infection, but no subsequent DNA synthesis. Furthermore, when cells infected with FST-DNA only are first allowed to synthesize proteins, superinfecting phages no longer require protein synthesis to transfer their whole DNA (Lanni, 1965). These latter observations suggested that the FST-DNA might be responsible for at least two functions: degradation of the host DNA, and synthesis of one or more proteins necessary for transfer of the rest of the viral DNA molecule.

Recently we have isolated amber (*am*) mutants and found that some of these were in two genes in the FST-DNA, while five other tested genes were allocated to the rest of the DNA molecule (Lanni *et al.*, 1966). This confirms the specificity of the FST-DNA and the homogeneity of its genetic content. We have now mutants in at least five sites in gene I and two sites in gene II. In the past few months we have confirmed that mutants in gene I, infecting a non-permissive host, can neither degrade the host DNA, nor transfer more than their FST-DNA; mutants in gene II cause normal host DNA degradation, but do not appear to transfer beyond the FST-DNA. These results thus confirm the functions previously attributed to the FST-DNA.

Dr. Juergen Weimann,
Divisional Technical Officer

Dr. D.J. McCorquodale has found that, after complete transfer of wild-type T5 DNA, bacterial protein synthesis is cut off after a short time, and three classes of phage-induced proteins are synthesized (McCorquodale et al., 1967). Partial infection by wild-type FST-DNA results in synthesis of class I proteins only, and this synthesis is cut off at approximately the same time as bacterial protein synthesis. Cut-off of class I protein synthesis does not occur in the presence of chloramphenicol, strongly suggesting that cut-off requires the synthesis of a new, FST-DNA-induced, protein.

Preliminary experiments (done in collaboration with Dr. D.J. McCorquodale) indicate that, after infection of a non-permissive host with mutants in gene I, only class I proteins are synthesized, one of the phage-induced proteins is missing, and cut-off of class I and bacterial proteins does not occur normally: synthesis of these proteins continues for a long time, though at a progressively reduced rate. Infection with mutants in gene II also results in class I protein synthesis only, followed, however, by a clear cut-off of both phage-induced and

bacterial protein synthesis. From these results we predicted that cells infected with mutants in gene I should remain superinfectable by mutants in gene I for a long time after the primary infection, if cut-off of class I protein synthesis does not occur, and that superinfection should result in synthesis of all three classes of protein, as well as phage production. On the other hand, cells primarily infected with mutants in gene II should only remain superinfectable until cut-off of class I protein synthesis occurs. Preliminary experiments indicate that this is so. These results confirm the previous findings: if mutants in gene I and II cannot transfer more than their FST-DNA to the nonpermissive host, one would not expect synthesis of class II and III proteins. In addition, the results indicate that we may have mutants in a gene normally responsible for the resulation of class I protein synthesis.

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W. MICHALKE

Orientation of DNA Synthesis in Bacteriophage T1

The replication of T1 DNA has been studied, following the method of Pratt *et al.,* (1961), by measuring the reversion of amber mutants to wild-type following different times of treatment with 5-bromodeoxyuridine (BUDR). Bacteria grown in BUDR were multiply infected with T1 phage in the presence of BUDR. Samples were then diluted at different times into thymidine medium and further incubated until lysis.

The frequency of revertants started to increase about 5 minutes after infection at 30°, 35° or 40°C. At 25°C the first increase in revertant frequency was observed at about 8 minutes. Probably no DNA synthesis occurs before these times, but a later start of DNA synthesis cannot be excluded.

After the start of DNA synthesis, the revertant frequency increases linearly with time of treatment for at least 8 minutes, depending on the temperature. Again the slope of this increase is about the same in the temperature range from 30° to 40°C and is less at 25°C.

The question whether two different points of a DNA molecule start to replicate at different times was tested by mutagenic treatment of double amber mutants at 40°C and measurement of the revertants at both sites. No significant time delay in the start of replication of two different sites was observed, indicating either that more than one starting point of DNA replication exists on the T1 chromosome or, more likely, that one replication cycle of the T1 chromosome takes less than about 20 seconds.

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M.H. PATRICK

The Nature of Repairable Damage in DNA

UV photochemistry of DNA. A third thymine-derived product in UV-irradiated DNA has recently been reported (Varghese and Wang, 1967). While the structure of this product is not yet known, it has been shown to have an absorption maximum around 312 nm. Irradiation at this wavelength will reverse the product to a compound chromatographically identical with thymine. We are investigating the biological significance of this product, using radioactive thymine- and cytosine-labelled DNA and biological assay of irradiated transforming DNA, subsequent to treatments such as enzymatic photoreactivation. Particular emphasis is being focused on *Streptomyces griseus* spores, in which a mutant has been found by H. Takebe that lacks the capacity for photoreactivation at the usual longer wavelengths, but can photo-reverse lethal far-UV damage with light around 312 nm.

It has been reported that thymine dimers can be formed in DNA by energy transfer from triplet state excitations in certain types of organic solute molecules, when these are exposed to wavelengths lying above the limit of DNA absorption (Lamola and Yamane, 1967). We have tentatively confirmed that aqueous solutions of DNA irradiated at 313 nm in the presence of low concentrations of the chemical sensitizer, acetophenone, yield a product chromatographically identical to thymine dimer. In contrast to the usual 254 nm UV irradiation (no sensitizer), there is apparently little or no formation of other thymine-derived products. In the absence of acetophenone, this product can be quantitatively reversed to one having the Rf of thymine, by exposure to 254 nm light.

Repair of damage in bacterial transforming DNA. H. influenzae possesses an efficient dark-repair mechanism for UV damage to DNA (Patrick and Rupert, 1967). In transforming DNA, the widely differing UV sensitivities among various genetic markers appears to reflect the extent to which they undergo dark repair. Presently being investigated is the correlation of extent of repair with extent of formation of a particular UV photoproduct. Using the acetophenone sensitization system described above, initial results show that: (1) differences in inactivation rates between markers, as well as the extent of dark repair, is significantly less for sensitized inactivation than for 254-nm inactivation, and (2) such damage is repairable by enzymatic photoreactivation. If the damage produced in the presence of acetophenone is indeed only thymine dimerization, such studies may provide a way to estimate more accurately the contribution of thymine dimer to biological inactivation and to assay the efficiency with which it is repaired.

Also in progress are attempts to determine the degree of dark repair of various damages in DNA. We have found that interstrand covalent cross-links induced by nitrous acid are dark-repairable, although to a somewhat less extent than such damage caused by UV.

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C.S. RUPERT, R. DAY, HELGA HARM

Inactivation and Repair of Transforming DNA

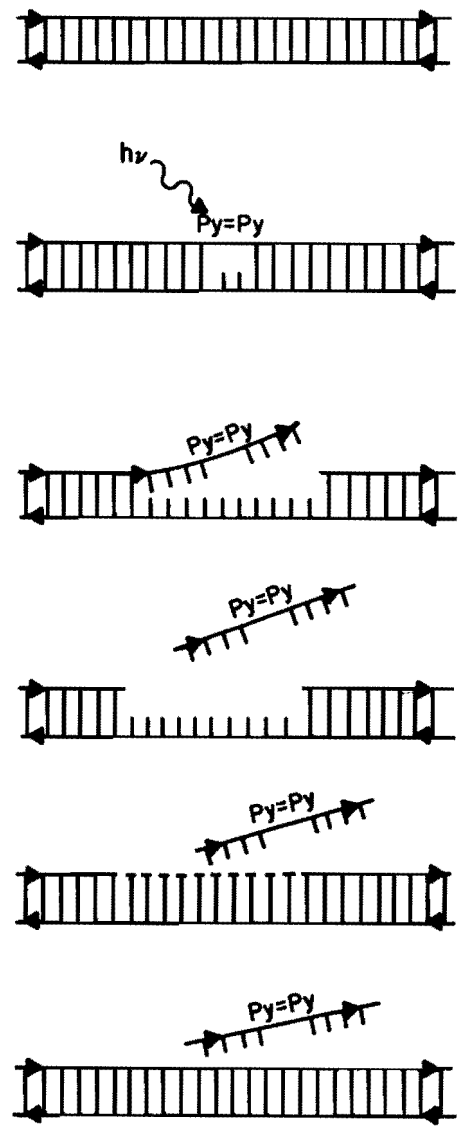
Inactivation of transforming DNA by chemical or photochemical injury. A theoretical investigation of models for the transformation process was undertaken to elucidate the shape of inactivation curves for linked-double markers in transforming DNA (i.e., markers lying on the same DNA fragments in the DNA preparation). A model correctly relating the fraction S of transformations surviving a dose D of UV radiation or nitrous acid for single markers [$S = 1/(1+CD)^2$], also gives the correct relation for linked-double markers [$S \approx 1/(1+CD)^4$], providing that random recombinations of the incoming DNA with the bacterial genome in the region between the markers is allowed for.

However, it was shown quite generally that other models differing from this one could also give the inverse-square law for singles and inverse fourth power for linked-doubles. Thus the ability of a model to predict these laws is not strong evidence for its validity. Nevertheless, in all the model types covered, the number of lesions per unit length of DNA was proportional to dose, so that ($\sqrt{1/S}$ -1) for single markers and ($\sqrt[4]{1/S}$-1) for loosely linked doubles would be a relative biological measure of the amount of effective damage.

Factors in the transformation process affecting UV sensitivity. A complex genetic marker Nb_2 of *Haemophilus influenzae,* conferring resistance to 50 μg/ml novobiocin, has been resolved into two closely-linked mutations Nb_1 and Nb_3 which can be scored separately as resistance to 5 μg/ml and 1 μg/ml respectively. Using cells carrying one or the other or neither of these mutations as recipients, the UV sensitivities of differently marked transforming DNAs were tested, scoring for the transformations corresponding to various possible recombinants of these two markers. It was found that wherever the drug-resistance phenotype selected for was one requiring a recombination between a marker in the cell and one on the incoming DNA (thereby decreasing the effective length of the transforming DNA strand actually utilized in the transformation) a diminished UV sensitivity of the DNA (dose-reduction factor $\approx$ 0.2) was observed. This result, consistent with the explicit model referred to in paragraph 1, rules out certain other possible models. On the other hand, when there was a mismatch in the Nb_3 region between the recipient cell genome and the incorporated segment of the DNA, an increased UV sensitivity (dose-modification factor $\approx$12) occurred. This effect is not shown for mismatches with respect to Nb_1. The two sensitivity effects are displayed independently, making the UV survival curves fall into four clearly defined groups.

Qualitatively the same effects are seen when using an ultraviolet-sensitive mutant of *H. influenzae* (colony forming D_{37} = 2 ergs/mm^2) as the recipient cell.

Flash photolysis of photoreactivating enzyme-substrate complex. Using high enough ratios of enzyme to UV lesions in irradiated transforming DNA, we are able to erase over 60% of the effective UV dose to the DNA with a single millisecond high intensity flash. A second flash then adds either little or much more dose reduction depending on whether sufficient time is allowed for the enzyme freed by the first flash to diffuse and attach to new damage sites (substrate). Thus, where a single flash erases 61% of the UV dose, a simultaneous second flash raises this to 68%. However, delaying the second flash by 1 second raises this to 74%, by 10 seconds to 79% and by 60 seconds to 80.5%. (The maximal dose reduction after complete photoenzymatic repair is close to



Excision-resynthesis repair (cut and patch) in DNA

90%.) The time required for a released enzyme molecule to "find" a new site is thus relatively long. The same conclusion is indicated when delayed flashes are applied after the irradiated DNA and PR enzyme are rapidly mixed. These effects are being used to probe time relations in the repair reaction.

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H. WERBIN

Photochemical and Photobiological Studies of Reactivation

Repair of UV radiation damage in blue-green algae. Algae have no doubt evolved mechanisms for simultaneously utilizing the visible rays of the sun for driving their photosynthetic machinery while counterbalancing the harmful UV radiation. One process for reversing harmful UV effects is photoreactivation and this phenomenon was recently found to occur in the blue-green alga, *Plectonema boryanum* (Wu *et al.*, 1967). The alga repairs far-UV damage to itself and a UV-irradiated phycovirus, LLP-1, which infects it. We sought a photoreactivation enzyme in extracts of *P. boryanum* in order to establish the molecular basis for this phenomenon in blue-green algae. Our efforts were successful (Werbin and Rupert, 1968).

Evidence that supports the enzymic nature of the active factor in algal juice is: (1) heat and addition of trypsin inactivate the factor, (2) the factor is precipitable by acetone and can be adsorbed to calcium phosphate gel, and (3) the factor is inactive at 0^{o}. Furthermore, the extent of photoreactivation is dependent on the dose of light and the concentration of protein in the algal extract.

Photochemistry of naturally occurring quinones. Although near-ultraviolet radiation (300–400 nm) is harmful to biological organisms and certain of their substructures, it can reduce the lethal far-ultraviolet effects in many micro-organisms (Jagger and Stafford, 1965). The cellular quinones, vitamin K, ubiquinone, and plastoquinone, have been implicated as the principal targets of near-UV radiation, but the precise photochemical alteration in molecular structure remains obscure.

To mimic the action of near-UV radiation on tri-substituted 1, 4-p-benzoquinones, such as plastoquinone and demethyl vitamin K_1 (found in plastids and in *H. parainfluenzae*, respectively) we chose as a model 2-methyl-1, 4-naphthoquinone (menadione). Sunlight irradiation of menadione in the solid state confirmed that the major products are two stereoisomeric cyclobutane dimers (Asahi, 1965). The dimers were easily detected by thin-layer chromatography on silica gel and were separated in macro quantities by column chromatography on a silicic acid-celite mixture. The same photo-dimers were produced by near UV irradiation of a microfilm of menadione on a glass slide.

In contrast, irradiation of menadione adsorbed to silica gel leads to a plethora of photoproducts detectable by thin-layer chromatography. Two of these appear to be the third and fourth cyclobutane photo-dimers.

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H.L.K. WHITEHOUSE

The Mechanism of Crossing Over

The model. Since the model was first proposed (Whitehouse, 1963, 1964), it has been modified and more clearly defined by postulating (a) that there are fixed primary breakage points in the DNA, and that these are located at the ends of genes (Hastings and Whitehouse, 1964), (b) that these opening points are at one specific end of each structural gene (Whitehouse, 1966), and (c) that the initial step in recombination is not breakage of a nucleotide chain but failure to make the phosphodiester link between one replicon and another after the previous DNA replication (Whitehouse, 1967a). The published fungal recombination data were found to be in agreement with the model (Whitehouse and Hastings, 1965), apart from some data associated with reciprocal recombination of alleles. These anomalous results are now attributed to a modification of the normal process of crossing-over due to multisite mutations (Whitehouse 1967c).

Application of the model has led to a picture of possible organization of DNA in the chromosome (Whitehouse, 1967a), and to a possible explanation of antibody variability (Whitehouse 1967b).

Testing the model using ascospore color mutants. An ideal situation for the study of recombination is provided by certain 8-spored ascomycete fungi. Asci (meioses) can be selected in which recombination has occurred between allelic mutants affecting the color of the ascospores. The recombinant asci can be recognized because they contain one or more wild-type spores. For the fullest information, it is necessary to have marker genes linked on each side to that for spore color. The studies with *Ascobolus immersus,* initiated by Rizet and associates, have lacked such markers, and those by Olive and associates with *Sordaria fimicola* have involved only a single ascospore color mutant. In collaboration with M.H.V. Cooray, D.J. Bond and A.F. Ahmad at the University of Cambridge, an investigation is being made of recombination at a particular gene for spore color (locus 2A, buff) in *S. brevicollis.* With the cooperation of Dr. L.S. Olive at Columbia University and Dr. W.G. Fields at Michigan State University, at least 15 mutants are now available at this locus, as well as linked marker genes on each side. Future experiments are expected to provide detailed information about recombination in this region of the chromosome. The results should be most useful as a test of the recombination model and, more indirectly, of the chromosome model.

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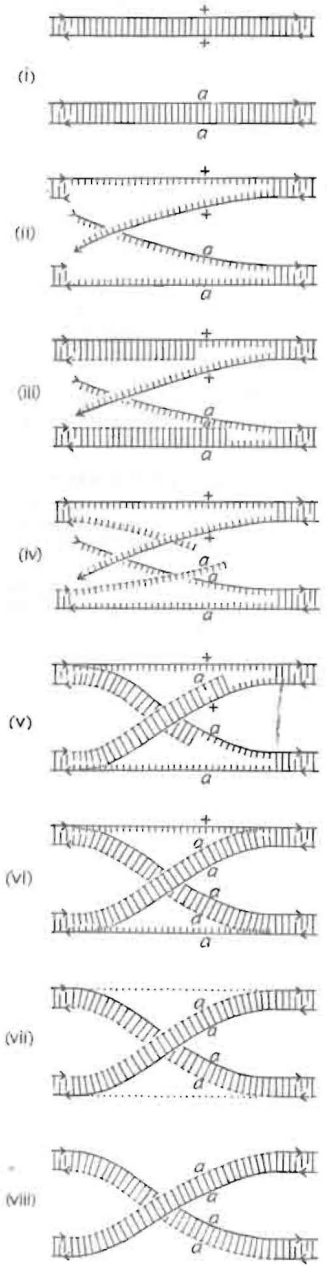
Harold L.K. Whitehouse



Mechanism of crossing over according to the Whitehouse-Hastings model

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Invited Seminar Speakers

DR. EDMUND BECKER, Science and Engineering Institute, Waltham, Mass.
"Polyamine synthesis in *E. coli:* effects of decarboxylaselessness"

DR. JAMES BELLI, Southwestern Medical School, Dallas
"Radiation damage repair in mammalian cells"

DR. ROLF BENZINGER, Institute of Molecular Biology, Geneva
"Host-controlled restriction of infectious single- and double-stranded DNA of bacteriophage *fd*"

DR. WILLIAM BRAMMAR, Stanford University
"Frame-shift mutations and amino-acid sequences in tryptophan synthetase 'A' protein of *E. coli*"

DR. B.A. BRIDGES, MRC Radiobiological Research Unit, Harwell, England
"UV-damage leading to bacterial death, true reversion and suppressor mutation"

DR. ROBERT BRUNER, University of California Berkeley
"Analytical-band centrifugation in self-generating density gradients"

DR. C.F. BRUNK, Stanford University
"Repair of U.V. and X-ray damaged DNA in *Tetrahymena pyriformis*"

DR. ARTHUR CHOVNICK, University of Connecticut, Storrs
"Studies on genetic organization in higher organisms"

DR. F. CHYTIL, Southwest Foundation for Research, San Antonio
"Activation of a hormone-induced enzyme"

DR. WILLIAM CRAMER, University of California, San Diego
"Spectrophotometric positioning of two cytochrome-*b* components in the electron-transport system of spinach chloroplasts"

DR. RAYMOND DEVORET, Yale University
"Indirect ultraviolet induction of λ prophage"

DR. J. FRANKEL, State University of Iowa, Ames
"Cortical differentiation and the cell cycle in ciliates"

DR. STANLEY GARTLER, University of Washington, Seattle
"Studies of X-chromosome inactivation"

DR. STUART GLOVER, MRC Microbial Genetics Research Unit, London
"Host-controlled modification in bacteria"

DR. SOL GOODGAL, University of Pennsylvania, Philadelphia
"Mechanism of integration of single stranded DNA of *H. influenzae*"

DR. JOSEPH GREENBERG, Palo Alto Medical Research Foundation
"Genetics of radiation resistance in *E. coli*"

DR. KARL GRELL, University of Tübingen, Germany
"Studies of the biology of suctoria"

DR. EDWARD GRULA, Oklahoma State University, Stillwater
"Bacterial cell division"

DR. PHILLIP HANAWALT, Stanford University
"Non-conservative DNA replication"

DR. ROBERT HAYNES, University of California, Berkeley
"Genetic implications of DNA repair"

DR. MARTIN HEISENBERG, California Institute of Technology
"RNA-bacteriophage formation *in vivo*"

DR. RAINER HERTEL, Michigan State University, East Lansing
"The geotropic reaction in plants"

DR. ANTON HEYN, Louisiana State University, New Orleans
"X-ray analysis of biological molecules"

DR. BRUCE HOLLOWAY, University of Melbourne, Australia
"Recombination and linkage experiments in *Pseudomonas aeruginosa*"

DR. SHEILA HOWARTH, University of Otago, New Zealand
"Modified effects of UV-irradiation on colicinogenic bacteria"

DR. DAVID HSIA, Childrens' Memorial Hospital, Chicago
"Biochemical changes associated with chromosomal abnormalities"

DR. T. IINO, National Institute of Genetics, Japan
"Morphogenesis of bacterial flagella"

DR. CLARENCE KADO, University of California, Berkeley
"Genetics of tobacco-mosaic virus"

DR. JOST KEMPER, Cold Spring Harbor Laboratories
"A new type of suppressor: gene and mutation specificity"

DR. HANS KOSSEL, University of Wisconsin, Madison
"Nonsense codons and chain termination in protein biosynthesis"

DR. HEINRICH KROEGER, Technische Hochschule, Zurich
"Regulation of gene activity in metazoan cells"

DR. ROBERT KROOTH, University of Michigan, Ann Arbor
"Genetic control of pyrimidine biosynthesis in cultured human diploid strains"

DR. SANFORD LACKS, Brookhaven National Laboratories
"Molecular fate of DNA in the genetic transformation of Pneumococcus"

DR. J.T. LETT, Chester Beatty Research Institute, London
"Repair of X-ray damage to the DNA of *Micrococcus radiodurans*"

DR. MYRON LEVINE, University of Michigan, Ann Arbor
"Replication and lysogenization with phage P22"

DR. MARGARET LIEB, University of Southern California, Los Angeles
"Studies of the λ repressor"

DR. D.J. McCORQUODALE, Emory University, Atlanta
"Patterns of protein synthesis in T5-infected *E. coli*"

DR. PAUL MOORHEAD, Wistar Institute, Philadelphia
"SV40-transformation of human cells; a model of malignancy"

DR. A.R. MORGAN, University of Wisconsin, Madison
"A highly specific DNA-RNA three-stranded structure and its possible biological significance"

DR. ANWAR NASIM, Atomic Energy of Canada, Chalk River, Ontario
"The induction of complete and mosaic mutants by mutagenic treatment of single cells"

DR. RAY OWEN, California Institute of Technology
"The immunological imperative"

DR. HERBERT POHL, Oklahoma State University, Stillwater
"The use of non-uniform electric fields in cellular studies"

DR. ROBERT ROMIG, University of California, Los Angeles
"Transformation and transfection in *B. subtilis*"

DR. JOHN SCAIFE, Harvard Medical School
"The promoter, a site controlling maximal gene expression"

DR. J. SCHELL, University of Ghent, Belgium
"Host-controlled restriction in *Escherichia coli*"

DR. ANTONIO SICCARDI, Stanford University
"Infective colicin resistance associated with R factors"

DR. A.S. SIDEROPOULOS, University of Kansas, Lawrence
"Mutational synergism between sub-lethal u.v.-irradiation and methylated purines"

DR. SIMON SILVER, Washington University, St. Louis
"Chemical and viral-induced changes in the cell membrane of *E. coli*"

DR. P. SITTE, University of Freiberg, Germany
"Isolated nuclear envelopes"

DR. FRANK STAHL, University of Oregon, Eugene
"Gene clusters in phage T4"

DR. BERNARD STRAUSS, University of Chicago
"Repair of alkylation and U.V. damage in *Bacillus subtilis*"

DR. ELDON SUTTON, University of Texas, Austin
"Chemical and genetic studies of mammalian transferrins"

DR. WACLAW SZYBALSKI, University of Wisconsin, Madison
"Mapping of the transcribing regions on the complementary strands of phage DNA"

DR. STEPHEN TAUB, Princeton University
"The determination and expression of mating type in paramecium: A problem of nuclear differentiation"

DR. HERBERT TAYLOR, Florida State University, Tallahassee
"Regulation of DNA replication at the cellular and molecular levels"

DR. J.R. TOTTER, U.S. Atomic Energy Commission, Washington, D.C.
"Sequence of chemical changes during chemiluminescence of phalazinediones and biacridines"

DR. JOHN TUCKER, Indiana University, Bloomington
"The fine structure, function and morphogenesis of a contractile microtubular organelle in the ciliate *Nassula*"

DR. ROBERT WAGNER, University of Texas, Austin
"The role of mitochondria in amino-acid synthesis"

DR. ROBERT WELLS, University of Wisconsin, Madison
"DNA-like polymers with defined, repeating nucleotide sequences"

DR. RUDOLF WERNER, Cold Spring Harbor Laboratories
"The number of replication sites in T4-infected bacteria"

Televised seminar (Dr. Sanford Lacks) in progress

Second Floor Layout, Founders Building

Staff of the Biology Division

1987

ACADEMIC

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Lucy Howrey
Michiko Mitani
Gert Wuesthoff

NON-ACADEMIC

Technical

Paula Adams*
Martha Astor
Ina Atkins*
Raymond Blake
Francis Burke
Barbara Coval*
John Denson
Rachael Flores*
Ola Fox
Pat Hall*
Mary Jo Harrod
Judith Hays
Elizabeth Hickman
Janet Humphrey
Alma Jimenez
Cheryl Kimble
Petra Klose
Kay LaRue
Joan Martinek
Esperanza Martinez
Beverly Moody*
Eric Moody*
Gretel Mueller
Genevieve Paule*
Deanna Robbins
Elizabeth Roeder
Phillis Shaw
Jeanne Snow
Ute Striebeck
Lynette Terrell
Maria Turner†
Carolyn Waghorne*
Lois Wilson
Brigitte Wolff
Dorothy Yuan

Administrative

Jeanne Thweatt
Jean Mormino
Renate Altmann
Pat Carlson†
Sue Clark
Jan Cooper*
Moreen Higgs
Kay Petty
Mary Zachary*

Media Kitchen

Cleo Bell
Larry Carbine
Alfreda Johnson
Beatrice Johnson
Gertrud Wiemann*

Instrument Shop

Hans Bank
William Bridenbaker*
Theodore Edmunds*
Robert Grissom
Elmer Pittsinger

*Left during the year
†Part-time

Vincenzo Coronelli's globe (circa 1686) shown at left above, may be the first map to show the approximate location of the Mississippi River. The globe, loaned to the Center by Robert E. Moody of Dallas, and the painting "Te Creces y Te Ausentas," at right below, are among permanent exhibits in our Founders Building. The painting, by Rafael Canogar, was given to the Center by the Mr. and Mrs. Stanley Marcus Foundation. Regional art exhibits are frequently on view in the lobby and corridors, through co-operation with art societies, art departments, and individual painters and sculptors.

Educational institutions in the Dallas-Fort Worth area