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Received Date	11-6-58
Council Assigned	Interim
Action	

Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH

Mail Completed Application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

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C-4496
GENETICS (7)
Formerly
C-2157 (C10)

APPLICATION FOR RESEARCH GRANT

Date November 1, 1958

Application is hereby made for a grant in the amount of $15 410 (omit cents) for the period from February (month) 1 (day) 1959 (year) through August (month) 31 (day) 1959 (year), inclusive for the purpose of conducting a research project entitled (Limit to 53 typewriter spaces).

GENETICS OF BACTERIA

Check One:
- [X] NEW PROJECT
- [ ] RENEWAL OF PHS GRANT NO.
- [ ] SUPPLEMENT TO PHS GRANT NO.
- [ ] REVISION OF PHS APPLICATION NO.

Principal Investigator		Co-Principal Investigator, if any:	
Name (First) (Middle) (Last)	Joshua Lederberg	Name (First) (Middle) (Last)	-----
Title	Professor of Genetics	Title	
Dept.	Genetics	Dept.	
School	of Medicine	School	
University or Institution	Stanford University	University or Institution	
Street Address		Street Address	
City and State	Stanford, California	City and State	
Name, Title and Address of Financial Officer:	Duncan I. McFadden, Controller Stanford University Stanford, California	Check to Be Drawn as Follows:	Stanford University

AGREEMENT

It is understood and agreed by the applicant: (1) That funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes set forth in this application; (3) that all reports of original investigations supported by any grant made as a result of this request shall acknowledge such support; (4) that, if any invention arises or is developed in the course of the work aided by any grant received as a result of this application, the applicant institution will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with its own policies, as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

NAME OF INSTITUTION: Stanford University

ADDRESS: Stanford, California

CITY AND STATE:

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION (*Please Type*): Frederic O. Glover, Assistant to the President

PERSONAL SIGNATURE (This agreement must carry the actual signature of the official whose name appears on the line above).

(use ink)

PRIVILEGED COMMUNICATION

PHS 398
Rev. 9-57

Form Approved
Budget Bureau No. 68-R249.7

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PROPOSED BUDGET for the period shown on page 1

NOTE: Under column entitled "OTHER" indicate funds presently available or anticipated from other sources, including those from own institution.	PERCENT OF TIME TO BE SPENT ON THIS PROJECT	BUDGET: REQUESTED FROM PHS (Omit Cents)	BUDGET: OTHER
PERSONNEL: Itemize All Positions, Indicating Type, Percent of Time To Be Spent On This Project and Names of Professional Personnel Selected.		$	$
J. Lederberg Professor Principal Investigator	60	----	Stanford Univ.
Research Associate, Ph.D.	100	----	6 000
Research Assistant, M.A. (* 7 mos. salary; in-	100	2800*	
Preparator B.A. (cludes OASI, retire-	100	2100*	
Dishwasher(s) (benefits where appl.)	100	1100*	
TOTAL PERSONNEL:		TT 6 000	
PERMANENT EQUIPMENT (See instructions reference itemization of equipment)		$	$
Res. microscope, ph. contr. + fluorescence accessories; camera; micromanipulator		2400	
Lyophil apparatus for culture preservation		700	
Incubators and water baths		1000	
TOTAL EQUIPMENT		TT 4 100	
Capital construction and other equipment of laboratory			85 000
CONSUMABLE SUPPLIES (Itemize)		$	$
Chemicals and glassware, including initial stocks		3 000	
TRAVEL (State Purpose) (U.S.)		$	$
To scientific meeting and other laboratories (prof. pers)		300	500
OTHER EXPENSE (Itemize) Publication		$ ---	$

NOTE: The administrative official signing this application may add an amount for indirect costs.

IMPORTANT

Review detailed instructions before computing indirect cost allowance.

SUBTOTAL (DIRECT COSTS)	$ 13 400
INDIRECT COSTS PHS PARTICIPATION ADJUST TO LOW DOLLAR	$ 2 010
TOTAL BUDGET (OMIT CENTS)	$ 15 410

ESTIMATE OF FUTURE YEARS REQUESTED FROM PUBLIC HEALTH SERVICE

ADD'L YEARS	PERSONNEL	EQUIPMENT	SUPPLIES	TRAVEL	OTHER	SUBTOTAL (DIRECT COSTS)	INDIRECT COST ALLOWANCE	TOTAL
1st	$ 10 000	$ 1 000	$ 1 000	$ 300	$ 200	$ 12 500	$ 1 875	$ 14 375
2nd	10 000	1 000	1 000	300	200	12 500	1 875	14 375
3rd	10 300	1 000	1 200	300	200	13 000	1 950	14 950
4th	10 300	1 000	1 200	300	200	13 000	1 950	14 950

If additional years requested are not contemplated enter "NONE" under total for first additional year.

PUBLIC HEALTH SERVICE SUPPORT: Show previous and current Public Health Service grants supporting this project:

GRANT NUMBER	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
PREVIOUS C-2157 to C9 to University of WISCONSIN	Genetics of Bacteria	10 yr total $83 065	1948-1958
CURRENT C-2157 C10 to University of Wisconsin	Genetics of Bacteria. The present application is for an equivalent amount (including carryover from C9 to be made available at Stanford U.)	13 800	1958-59

ALL OTHER SUPPORT: Excluding Public Health Service, but including that from own institution, list support from other sources for this project. If none, so indicate.

SOURCE	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
CURRENTly committed: Stanford Univ.	Capital construction; Dept of Genetics salaries and operating costs	~$ 150 000	1959
PENDING applications for additional funds: NSF	Genetic recombination in bacteria	5 yr total $ 109 500	1959-1963

RESEARCH PLAN AND SUPPORTING DATA

On the continuation pages provided give details of the proposed plan and other necessary data in accordance with the outline below. Number each page, the first continuation page being page 4. Additional continuation pages, if needed, may be requested from the Division of Research Grants. See detailed instructions before preparing this portion of the application.

1. RESEARCH PLAN
   - A. Specific Aims—Provide a concise statement of the aims of the proposed work.
   - B. Method of Procedure—Give details of your research plan. For each specific aim mentioned in "A" show how your plan is expected to fulfill the aim.
   - C. Significance of this Research—Explain why the results of the proposed work may be important.
   - D. Facilities Available—Describe the general facilities at your disposal. List the *major* items of permanent equipment.
2. PREVIOUS WORK DONE ON THIS PROJECT
   Describe briefly any work you have done to date that is particularly pertinent.
3. PERSONAL PUBLICATIONS
   Cite your most important publications on this or closely related work. List no more than five.
4. RESULTS OBTAINED BY OTHERS
   Summarize pertinent results to date obtained by others on this problem, citing publications deemed pertinent. Select no more than five.
5. BIOGRAPHICAL SKETCHES
   Provide brief sketches for *All* professional personnel selected who are to be actively engaged in this project.

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RESEARCH PLAN AND SUPPORTING DATA

1A-B. Current work and research plans.

On the whole, we plan to continue our studies of mechanisms of genetic transfer in bacteria. For the most part, these are direct extensions of current work on sexual recombination in Escherichia coli and on transduction by phage in E. coli and Salmonella. Past accomplishments are summarized in the comprehensive summary appended hereto. It is not feasible to separate consideration of recent findings, current operations and future plans.

The following aspects can be expected to have preferential attention by the group of students and associates working in the laboratory. (I may add at this point that Mrs. Lederberg's participation has facilitated the management of a larger program than might otherwise be possible. I can therefore plan to spend the larger part of my own time in the laboratory. The students and fellows here are responsible for the main research activity. Much of the technical help we require is to handle routines of medium-making and general housekeeping for the common benefit of students and senior investigators. These routines are quite extensive in the type of work we do.)

a. The nature of the F compatibility factor and its relationship to Hfr loci. This study will involve the further analysis of a series of Hfr mutants already isolated. Some of the Hfr's have demonstrably different locations, but do not seem to involve rearrangements of other bacterial markers, an important point in various hypotheses of F/Hfr relationships now current. Another approach is the inheritance of F in matings, both en masse and in single cell pedigrees. F seems to differ from all other markers in its contagiousness, as it will spread throughout an F- culture seeded with a single F+ cell. However, more detailed studies of this are needed. A plausible working hypothesis, which differs slightly from that advanced by Jacob and others, is that the F+ mating type carries the F agent as a cytoplasmic factor, while the same agent can become fixed to various chromosomal sites to give Hfr types. Mr. Y. Hirota has discovered that the treatment of F+ cultures with acridine orange results in F- types. Preliminary experiments in microdroplets verify that this is an induced loss and not merely a selection against the F factor. More detailed studies are required to determine whether the dye merely inhibits the replication of the F factor or actually destroys it. Studies are also being continued on the conditions of its action, for example, on the identification of a cofactor which is found in peptone, as clues to its target in the F+ bacterium.

b. The well-known phenomenon of phase-variation of flagellar antigens in Salmonella has been analysed by genetic transduction methods, with the finding that a phase-determinant is linked to or identical with the H_2 (phase-2 antigen locus. This determinant oscillates between an active and inactive state. Further studies are directed at 1) the genetic control of this alternation, in monophasic variants, and 2) its possible control by environmental factors. Some preliminary experiments suggest that temperature shocks cause a slight phase shift, but it has not yet been possible to disentangle it from a possible differential killing of the two phases by heat.

A new approach to the problem has been furnished by the discovery of special strains of Salmonella that can be hybridized with E. coli. Dr. L. Baron's observations on this point have been confirmed and strains suitable for large scale investigation of Salmonella by sexual recombination techniques are being developed. These strains should also make it possible, for the first time, to correlate other aspects of the genetic control of specific functions in Salmonella by transductional and by sexual recombinational analysis. For example it should become possible to estimate the precise scope of the individual act of transduction in relation to the entire map.

c. DNA-mediated transduction (transformation). The direct transfer of markers by DNA in enteric bacteria would be an invaluable tool in the advancement of genetic chemistry. In contrast to the pneumococcus and hemophilus, where genetic study for other reasons is more difficult, enteric bacteria have so far given negative or indecisive results in the hands of a number of investigators, myself included. My own past trials in this direction have been relatively casual. The technical problem has become so urgent that a more concerted effort is now called for. Since 'dna' transferred by phage particles is genetically effective, the main impediment to dna-transduction may be reasoned to be in the penetration of dna particles into the recipient bacteria. Some of the variables to be manipulated in this program are (1) the test marker, (2) the genotype and the strain of the donor cells (3) the method of preparation and the state of purification of the DNA, (4) conditions of application and pretreatment of the recipient cells, and (5) genotype and strain of the recipient. Existing information on the pneumococcus gives some possible empirical guideposts, but there is probably nothing better to do than trial and error, an approach that would hardly be commendable for a less urgent technical aim. As to (4) particular emphasis will be laid on the use of protoplasts and L-colonies as recipients, though this rationale has so far not been substantiated. As to (1 and 5) stress will be laid on markers which are transducible by phage with high efficiency, and on systems where recombination by other mechanisms is under precise control. However, rather than rely too heavily on a priori rationalizations, much weight will also be given to an empirical approach. One instance of such an approach was not successful: namely, a screening of some 200 distinct strains of E. coli as potential recipients.

New encouragement for success in this direction comes from the recent observation of Dr. A.B. Kaiser on the transfer of Gal^+ genes by DNA extracted from lambda. We look forward to a close association with Dr. Kaiser who will be working in an adjacent laboratory in the Department of Biochemistry at Stanford.

d. Physiology of mating. The various steps of mating in E. coli may be systematized as follows: collision and agglutination; conjugation, fertilization, chromosome synapsis and crossing-over, segregation. During the past several months, Dr. L. Cavalli (Pavia, Italy) collaborated with us in an experimental review and theoretical kinetic analysis of the experiments on interrupted fertilization published by Wollman et al., 1956. which are an indispensable basis for further studies in this field. A closer look at each step is now in order. For example, their analysis of interrupted fertilization is based on the extrapolation of time-dependent curves for the recovery of various markers. These curves are sometimes rather shallow and their detailed form difficult to analyse on account of the continued initiation of new matings in the cell mixtures.

For more precise kinetic analysis the various steps should be more exactly controlled by environmental factors. We were unable to separate collision from conjugation; at lower temperatures potential conjugal pairs do not accumulate. One approach to separating conjugation from fertilization was a 'pulse-mating' experiment: mating was permitted at very high cell densities for one minute; the mixtures were then gently diluted a thousand-fold to allow the progression of conjugal pairs already formed, but prohibit new pair formation. However at high densities, the rate of mating followed a square root rather than the expected second power dependence on total cell concentration. This partly frustrated the design of the experiment; it may be related to finding that extra female cells added to a mating tended to interrupt matings already in progress, suggesting some form of active competition for the active sites on male cells. A more promising lead was the finding that periodate in certain concentrations would temporarily demasculinize male cells, neither killing them nor interfering with the progression of matings already started. This strongly suggests that a periodate-sensitive carbohydrate is involved in the specificity of the initial mating reaction, and chemical comparisons of male and female cells are projected along with trials of various polysaccharases to try to test this supposition.

e. Cytogenetics of fertilization. (Mr. A. T. Ganesan). Apart from the initial demonstration of conjugal pairs classical methods have not been given their full due in the study of fertilization; mainly for want of assistance by suitably trained advanced student or fellow. Mr. Ganesan's background in yeast cytology and genetics (mainly at the Carlsberg Laboratory at Copenhagen) is most promising in this respect. The original photographs gave some hint of the passage of Giemsa-positive material but a critical analysis still has to be made. Closely connected with this will be efforts to assay the transfer of P^{32} labelled DNA from labelled male protoplasts mated to female rods by means of the micro-radiographic 'star' method of Levinthal. We have verified that male protoplasts retain their mating competence and that progressive fertilization can be interrupted without disturbing the female member by lysing the male conjugant in distilled water. The very few unlysed (dead?) males should be recognized by very high star counts; fertilized female cells which can be washed following enzymatic extractions, if needed, should have a star count reflecting the input of labelled DNA. This experiment should permit a final verification of the Jacob-Wollman hypothesis of progressive fertilization, and the correlation of quantity of DNA with genetic length. Our former facilities at Wisconsin were unpromising for this long-planned experiment; it may be done at Stanford or in collaboration with Dr. S. Lederberg of Brown University. Garen and Skaar have published experiments on P^{32} transfer in mass matings.

f. Recombinational analysis of galactose mutations. (E. M. Lederberg). The complex of closely linked Gal mutants affecting the fermentation of galactose occupies a promising place in biochemical genetic correlation for several reasons: (1) the identification of sequential defects in specific enzymes by Kalckar; (2) the scope and simplicity of analysis of these factors by "high frequency" transduction by the phage lambda; (3) the availability of more than one hundred nonrecurrent mutants. Many of the mutants fit into a simple picture, whereby a set of mutants falling into one cistron (position effect group) corresponds to one of the three enzymes (kinase, transferase, epimerase) in Kalckar's scheme. However, a number of anomalies have appeared, e.g., the mutant Gal_3 which behaves recombinationally as a point mutant, but impairs the formation of all three enzymes, and overlaps at least two of the cistrons; another mutant Gal_{22} belongs to neither of the other cistrons (i.e. forms galactose-positive transheterogenotes with each of them). The validity of the concepts of simple cistron-enzyme relationships (i.e. of linear coding) so facilely accepted by many workers today, needs to be tested vigorously and extensively. Some indication that Gal_3 is structurally aberrant has been found from experiments in which various Gal mutants are mapped by 'timing' in interrupted fertilization. Gal_3 is delayed several minutes whereas most of the Gal mutants fall within one minute of one another. The time-mapping, which requires considerable technical improvement to facilitate its use for short intervals, is also being applied to determine whether each cistron maps compactly without overlapping the loci of other cistrons. Other efforts to map the sequence of Gal mutants have occupied a great deal of our time during the past two years, but have been frustrated by a high coincidence of crossing-over in three and four point tests. Unfortunately, few known markers are closely linked to Gal; extensive surveys to find other auxotrophic markers that would accompany Gal in transduction by lambda have failed.

Parallel studies are under way with complexes of Lac (lactose) and Ara (L-arabinose) mutations.

g. Prophage relationships in lambda transduction. The findings by Campbell, Arber and others that auxiliary phage greatly increases the efficiency of transduction removes the main support for our previous conclusion that the same phage particle may carry the Gal markers and an intact phage. Studies on more complex systems (syngenotic recipients; and transductions to bacteria lysogenic for related phages) still leave open the possibility of at least an occasional association either in the original transduction, or in the reorganization of the input material in the heterogenote. These studies will be resumed in connection with the mapping of the exogenotic markers in heterogenote crosses, as mentioned above.

C-4496

1C. Significance of this research.

The principal motivation of this research program is further understanding of cellular heredity. Findings in bacterial genetics may also be expected to have an important bearing on connected areas of taxonomy, physiology, ecology, and so forth. Although we are not immediately concerned with practical applications in medicine, further improvements in medical practice must depend on our fundamental knowledge of etiological agents of disease. The analysis of genetics of microorganisms also plays a role in the comprehension of the genetics of viruses and of higher organisms and their component cells.

1D. Facilities available.

After a short interval of temporary occupancy of quarters in the Biophysics Building at Stanford University, this program will be housed in a group of laboratory rooms in the new medical center. We will be working in close association with the Department of Biochemistry and will share with them the use of, and responsibility for maintenance of many general facilities as well as highly specialized equipment. The basic items of equipment are being assembled from support from University and other sources as well as the present grant and we should be well equipped to recommence our research program soon after moving into the temporary laboratory February 1, 1959.

2. Previous work. See summary report appended.

3. Joshua Lederberg. 1947. Gene recombination and linked segregations in Escherichia coli. Genetics 32:505-525.

Joshua Lederberg, Esther M. Lederberg, N. D. Zinder and E. R. Lively. Recombination analysis of bacterial heredity. 1951. Cold Spring Harbor Symp. 16:413-443.

Joshua Lederberg, L. L. Cavalli, and Esther M. Lederberg. 1952. Sex compatibility in Escherichia coli. Genetics 37:720-730.

Esther M. Lederberg and Joshua Lederberg. 1953. Genetic studies of lysogenicity in Escherichia coli. Genetics 38:51-64.

Joshua Lederberg. 1956. Linear inheritance in transductional clones. Genetics 41:845-871.

4. Results obtained by others. Recombination genetics of bacteria is now too active a field to be summarized briefly. Various aspects are dealt with in recent symposia, e.g., Cold Spring Harbor Symposium for 1956, and the McCollum-Pratt Institute Symposium on the Chemistry of Heredity, Baltimore, 1956.

5. Biographical sketch.

Principal investigator - Joshua Lederberg, b. Montclair, N.J., May 23, 1925. B.A. (Zoology) Columbia College, 1944. Columbia University, College of Physicians and Surgeons (medical student) 1944-1946. Yale University, (Microbiology), 1946-1947, Ph.D. University of Wisconsin, Department of Genetics: Assistant Professor, 1947-1950; Associate Professor, 1950-1954; Professor of Genetics, 1954-1958; Professor and Chairman, Medical Genetics, 1957-1958.
STANFORD UNIVERSITY Medical School: Professor and Executive Head, Department of Genetics, 1959------.
University of California, Berkeley. Visiting Professor of Bacteriology, Summer, 1950. Melbourne University (Australia). Fulbright Professor of Bacteriology, Spring 1957.

6. Justification of specific budgetary requests.

This application is for the resumption at Stanford University of the program that has been undertaken at the University of Wisconsin with public Health Service support under grant C-2157 (C10). The budget contemplates a program of the same scope as has been supported by the NIH since 1952 taking account of increases necessary on account of salary and cost advances. For several years we have had collateral support from the National Science Foundation and a request is pending to them for renewal at Stanford University. The request to NSF was enlarged in order to take account in part of the expiration of support from the Rockefeller Foundation and the Wisconsin Alumni Research Foundation. It may be necessary at a later date to file a supplemental application to the NIH in order to continue the program at the level at which it has been carried out at Wisconsin but this is being deferred until we can better assess our needs at Stanford and the present application covers only those funds released by the termination of the project at the University of Wisconsin.

The principal investigator expects that the present project will continue to occupy the larger part of his time. Another request to the NIH is pending primarily for the support of Dr. Gustave Nossal in a program for the study of antibody formation in single cells.

Also pending is a request for a substantial graduate research training grant. If this is successful the support of graduate students as research assistants on the research program will be met from that grant with the exception of a small number of foreign nationals.

In anticipation of this transfer, many expenditures on C-2157 (C10) have been deferred and the personnel of the laboratory at Wisconsin has been kept to a minimum during the present year. In consequence the entire allocation from that grant for the current budget year will be available together with a small carryover from previous years. This, happily, may allow of the necessary initial capital expenditures needed to get the laboratory underway. The estimate for future years corresponds to the commitments already extended by the NIH for C-2157 (C11-C14). These estimates for future years contemplate a program of the same scope as that now being supported with a small allowance for increased costs by the third and fourth years.