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Council assigned Nov. '57
Action

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

APPLICATION FOR RESEARCH GRANT

Rec'd. May 27, 1957

(LEAVE BLANK)
C-2157 (C10)

Micro (2)

Former grant No. if different

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
DIVISION OF RESEARCH GRANTS
Bethesda 14, Maryland
PRIVILEGED COMMUNICATION

Date May 15, 1957

Application is hereby made for a grant in the amount of $ 13 800 (omit cents) for the period

from Sept. (Month) 1 (Day) 1958 (Year) through August (Month) 31 (Day) 1959 (Year)

inclusive for the purpose of conducting a research project on the following subject:

(LIMIT TITLE TO 53 LETTERS AND SPACES)

Title Of Project: genetics of bacteria

Name, Title And Address Of Principal Investigator:
Joshua Lederberg
Professor of Medical Genetics
Department of Genetics
University of Wisconsin
Madison 6 Wisconsin

Name, Title And Address Of Co-investigator, If Any:

Check To Be Drawn In Favor Of:
The Regents of the University of Wisconsin

Address:
171 Bascom Hall, University of Wisconsin
Madison 6, Wisconsin

Name, Title And Address Of Financial Officer
A.W. Peterson
Vice-President, Business & Finance
Bascom Hall, University of Wisconsin
Madison 6, Wisconsin

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: University of Wisconsin

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION (Please Type): A. W. Peterson, Vice President Business and Finance

x

C 2157

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

BAC

PHS 398
Rev. 7-56

Form Approved
Budget Bureau No. 68-R2497

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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 own institution.	BUDGET REQUESTED FROM P.H.S. (omit cents)	BUDGET OTHER
PERSONNEL (ITEMIZE ALL POSITIONS BY INDICATING TYPE; NAMES OF PROFESSIONAL PERSONNEL, IF SELECTED)		
Project Associates E.M. Lederberg, Ph.D.*		$ 5 000
?? P A Sneath or L L Cavalli		5 500
Project Assistant J. St. Clair, M.A.*	4 400	
Laboratory Assistant A. K. Ceak, B.A. *	3 800	
Graduate Student, 1/2 time	1 920	(4) 7 680
(* experienced personnel currently working)		
PERMANENT EQUIPMENT (Itemize) Addl. phase lenses; water baths; photo accessories	800	1 000
CONSUMABLE SUPPLIES (Itemize) Chemicals and glassware;	580	1 500
TRAVEL (State Purpose) Scientific meetings and research conferences	300	250
OTHER EXPENSE (Itemize) Reprints & Publication; laundry; service charges	200	200
SUBTOTAL (Direct Costs)	12 000	
NOTE: The administrative official signing this application may add an amount for indirect costs in accordance with the instructions. PHS PARTICIPATION IN INDIRECT COSTS (omit cents — adjust to low dollar)	1 800	
TOTAL BUDGET (omit cents)	13 800	

ESTIMATE OF FUTURE REQUIREMENTS—applies to funds needed from the Public Health Service for the years subsequent to the period proposed for this application. The spaces at the right are to be used to indicate the amount of support needed for each year, showing the direct and indirect costs as appropriate. DO NOT LEAVE ANY SPACES BLANK—If no additional support is required, enter "None". FOR FURTHER INFORMATION: See detailed instructions accompanying application form.

	DIRECT COSTS	INDIRECT COSTS	TOTAL
1	12 500	1 875	14 375
2	12 500	1 875	14 375
3	13 000	1 950	14 950
4	13 000	1 950	14 950

(LEAVE BLANK)

C-2157 (C10)

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 --C8	Genetics of Bacteria	$4000- 9775 per annum	1948- 1957
CURRENT			
C-2157 C9	Genetics of Bacteria (Request for supplement to accomodate salary increase is pending. Total amt:	13 800	Sept. 1 1957- August 31, 1958

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
CURRENT			
University	Genetics of Bacteria	10 500	July 1 1957 -June 30, 1958
NSF	Genetic transduction in Bacteria	7 500 per annum	Jan 1 1956 - Dec. 31, 1958
PENDING			
Rockefeller Foundn.	Genetics of Bacteria	6 500	Sept. 1, 1957 - August 31, 1958

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.

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. This line of work is outlined in detail in the cited publications, and in the progress report submitted in a companion application, for 1957-58, as well as 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 spend the larger part of my own time in the laboratory. The graduate students 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 correlation of lambda prophage with bacterial genes. This will involve further analysis of heterogenotes and heterozygotes which are segregating both prophage markers and mutant Gal markers of the bacterium. An intensive search is also being made for other prophage-linked markers. (Dr. E. Lederberg)

b. 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. Finally, Mr. Y. Hirota, a student at the University of Osaka, has discovered that the treatment of F+ cultures with acriflavine results in F- cultures. He is coming to complete his graduate studies here, and will study this effect further to determine whether it is a true induced loss of a cytoplasmic F factor or merely a selective effect. (Mr. Richter)

c. 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. (Mr. T. Iino).

d. 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. Current trials include the use of some 200 distinct strains of E. coli as potential recipients.

1C. Significance of this research.

Our research program is directed at fundamental analyses of cellular heredity. The significance of this work rests on the interest and validity of the scientific findings, such as those recorded in the appendix. The point hardly needs to be labored that such findings are basic to advances in medical application. Fortunately, this viewpoint no longer needs to be defended; it is, for example, the 'organic act' of the Department of Medical Genetics which has recently been established at the University of Wisconsin Medical School, (v.i.).

1D. Facilities available.

At the present time, this program is housed in a laboratory in the Department of Genetics, College of Agriculture. Except for chemical work and large scale culture, we are well equipped for the indicated studies, though somewhat cramped for space. For specialized needs, we have access to the resources of the Departments of Biochemistry and Bacteriology and the Enzyme and Cancer Research Institutes. For example, we are collaborating at present with Professor C. Heidelberger on the genetic effects of the uptake of bromouracil, fluorouracil and other analogues into bacterial DNA.

In about two years, (Spring, 1959) we expect to move into a new Research Wing of the School of Medicine, plans for which are now being drawn. It is not anticipated that this will cause any marked deviation either in the direction or the scope of this research program, although other members of the Department of Medical Genetics may be expected to be following their own lines of work in adjoining laboratories. Certain facilities, especially for chemical work, will be markedly improved.

2. Previous work. See progress 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.

In addition some outstanding contributions include:

Jacob, F. and E. Wollman (1956, 'Sur le processus de conjugaison et de recombinaison chez Escherichia coli.' Ann. Institut Pasteur, 91:486-510; also the Baltimore symposium, v.s.) have found that mating of compatible cells may be interrupted in course by agitation of the mixed cultures. When this is done at various times, the frequency of segregant types changes so as to suggest an orderly movement of a linear chromosome from one cell to the other. This chromosome may be broken by premature separation of the mates. (While other interpretations of the time sequence are not wholly excluded, this had been the principal methodological advance of the recent past in this field.)

Hayes, W. (1957, The kinetics of the mating process in Escherichia coli, J. Gen. Microbiol. 16:97-119) has confirmed many of Jacob & Wollman's results, and studied some of the environmental conditions of mating, and the timing of expression of recombinant genotypes. Krebs-cycle metabolites are necessary for mating to occur, possibly to furnish the energetic needs of the migratory gametic chromosome.

Cavalli, L. L. and J. L. Jinks (1956, Studies on the genetic system of E. coli K-12. Jour. Genetics, 54:87-112) have made the most elaborate

linkage mapping analyses of a large series of markers. The anomalies of segregation can be explained by the presence of an effective lethal (missing segment of a chromosome?) in every zygote, and an intermittent pattern of chromosome pairing.

Demerec, M. and collaborators (1956, Genetic studies with bacteria. Carnegie Institution Publication 612) have used transductional technique in Salmonella for the analysis of genetic fine structure. Genes affecting related biosynthetic functions, e.g. the synthesis of tryptophane, are often located in close proximity with one another, and even (according to these studies) in the same linear sequence as the chemical reactions themselves. These findings speak for a rationale of chromosome organization that has been quite unprecedented. In addition, mutations for the same function are often not identical, but closely linked. Each functional 'gene' is therefore a segment of the chromosome which can be altered at any of a number of component loci.

5. Biographical sketches.

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, 1954-1957; Professor of Medical Genetics, 1957- .....University of California, Berkeley. Visiting Professor of Bacteriology, summer, 1950. Consultant, Panel on Genetic Biology, National Science Foundation, 1953-1956. Consultant, Study Section on Microbiology, National Institutes of Health, 1957- .... Consultant, Committee on Growth, 1954. Member, National Academy of Sciences, 1957.--

Project Associate - Esther M. (Zimmer) Lederberg, b. December 18, 1922. B.A. Hunter College, 1942. M.A. (Biology) Stanford University, 1946. Ph.D., Wisconsin, 1950. Scholar, N.Y. Bot. Garden (mycology) 1941-42. Research Assistant (Carnegie Institution) at N.I.H., Bethesda, 1942-43. Junior Biologist, P.H.S., N.I.H., 1943-1944. University of Wisconsin, Department of Genetics; P.H.S. Predoctoral research fellow, 1947-1949; University fellow, 1949-1950. Project Associate in Genetics, 1950 --- .

6. Justification of specific budgetary requests.

The budget contemplates a program of the same scope as has been supported by the N.I.H. since 1952. An increase in budget to the current level on account of salary increases was applied for in a recent continuation application (for 1957-58) and the same level, $12,000 in direct costs, is embodied in the current application. The increases for subsequent years are in anticipation of further rises in academic salary levels, though these may prove to be larger than the 2-3% per annum indicated here.