September 1, 1958

RESEARCH PROPOSAL

to the

NATIONAL SCIENCE FOUNDATION

GENETIC RECOMBINATION IN BACTERIA

FOR A FIVE YEAR PERIOD, Jan. 1, 1959 - Dec. 31, 1963

Total funds requested: $ 109,535.

Submitted by

Leland Stanford Junior University
Stanford, California

Principal Investigator:

Joshua Lederberg
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[1] designate. Effective January 1, 1959.

ANNUAL BUDGET SUMMARY 1959-60-61-62-63

ITEM	- - - - - nsf - - - - - -			- other sources 1959 -	
	1959	1960	1961-2-3	PHS	Stanford
SALARIES					
Principal Investigator (research time prorata)	--	--	--	--	12,000
Research Associates (2)	3,000[1]	6,000	6,500	6,000	
Research Assistants (2)	2,000[1]	4,000	4,500	4,500	
Grad. Res. Assts. (4)	3,000	6,000	6,000		6,000
Other assistance					4,000
CAPITAL CONSTRUCTION and remodelling	--	--	--	--	50,000
EQUIPMENT laby instrumts.	3,000	1,500	1,500	1,000	5,000
SUPPLIED-Chem & Glassware miscellaneous	1,500	1,500	1,500	500	2,000
REPRINTS and other printing	250	250	250	200	
TRAVEL (princ. invest. & staff to meetings and lab. conf'ces)	500	500	500	300	200
SUBTOTAL	13,250	19,750	20,750	12,500[2]	79,200
ALLOWANCE for indirect costs (15%)	1,987	2,962	3,112	---	---
TOTAL	15,237	22,712	23,862 (x 3)	5 yr. total= $109,535	

1. These figures represent anticipated expenditures on salaries averaging one half of the first year to allow for delays in the establishment of the laboratory. The numbers in parentheses represent the entire staff; the present application would cover one research associate, one assistant, and two graduate assistants. The stipends for graduate assistants allow for tuition charges of $1,000 per annum leaving a net stipend of not more than $2,000. The liability of this stipend to income tax is also in question.

2. This figure reflects the level of 'approved anticipated future requirements' from the PHS for present grants to the University of Wisconsin for the period to August 31, 1963. This is subject to approval for 'transfer' of these funds for my use at Stanford. I anticipate the possibility of submitting application for supplemental funds from the PHS if this proves necessary to support the program as it develops.

The present request is not duplicated by any other application now anticipated.

Proposal: GENETIC RECOMBINATION IN BACTERIA
By: Joshua Lederberg, Professor of Genetics, Stanford University, California.
Funds Requested: $109,535 for five year period Jan. 1, 1959 to Dec. 31, 1963.

INTRODUCTION

Our principal intention is to pursue and expand lines of work already in progress. This centers on two phenomena of genetic recombination in Escherichia coli, sexuality and transduction. At various times in recent years, projects involving other systems have been developed in this laboratory. However, they are now being pursued elsewhere by the graduate students and fellows who collaborated on them here (cf. 28,59,60,71)

An important factor in our plans is the transfer of our work from Wisconsin to Stanford, further details of which will be given in current correspondence. Various students, associates and I may move from Wisconsin at various dates between January 1 and July 1, 1959, in time to occupy new quarters in the new Stanford Medical Center on the university campus. It will greatly facilitate the transfer of research activities if the grant is activated early in 1959 to allow for advance ordering of equipment and supplies and other expenses during the six months transition.

An important advantage of the Stanford situation is the close association it will afford with the Department of Biochemistry (headed by Professor Arthur Kornberg). Genetics will adjoin the Biochemistry and Pharmacology Departments, and substantial sharing of laboratory services and facilities is planned for. More important, Kornberg and his associates (including M. Cohn, P. Berg, D. Hogness and A. D. Kaiser) are actively interested in various aspects of microbiological chemistry and physiology and virology, closely intertwined with our own interests in bacterial genetics. We have not yet laid detailed plans for collaborative research efforts but can be certain of the strongest possible support in the expansion of biochemical aspects of our work, which hitherto have not had the attention they warrent.

RESEARCH PLANS

The following outline covers current projects intended for the next several years. It is, of course, impossible to make definitive long term plans in an area where vital new leads may arise quickly and unexpectedly. Various graduate students and fellows may have primary responsibility for pursuing different particular problems which are usually the subjects of dissertations. This particularization is indispensable to develop their individual sense of responsibility, but all the work in the laboratory is conducted with close consultation and supervision, and I will usually have been a manual collaborator during the early development of each problem.

1. Physiology of mating. (Dr. Peter Sneath) The various steps of mating in E. coli may be systematized as follows (52, A1): 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. (A1) 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 (cf. 52). 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 de-masculinize 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.

2. Cytogenetics of fertilization. (Mr. A. T. Ganesan). Apart from the initial demonstration of conjugal pairs (57) 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 promssing 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'1 method of Levinthal (A3). We have verified that male protoplasts retain their mating competence (70) 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 present facilities at Wisconsin are not promising for this long-planned experiment; it may be done either at Stanford or in collaboration with Dr. S. Lederberg of Brown University. Garen and Skaar (A3) have published experiments on P^{32} transfer in mass matings.

3. Non-Disjunctional Heterozygotes. Persistent diploids occur as rare exceptions in most matings, more frequently when one parent carries a 'Het' factor. Previous studies (44) had indicated that breakage of the paternal chromosome occurred uniformly at a given locus or loci; a reinvestigation suggests that the point of breakage can vary with the duration of fertilization, in accord with the Jacob-Wollman model. Our earlier results can be accounted for by the inept choice of selective markers. However, one anomaly still cannot be simply accounted for: maternal-deficient diploids. These

observations have to be consolidated before any theory of their origin can be tested. Our working hypothesis is that some spontaneous breaks are likely to occur in the paternal chromosome which result in a terminal deletion only after crossing-over. An exchange between the break point and a given marker, followed by the loss of the terminal segment will save the paternal allele and lose the maternal homologue.

Connected with this analysis is the observation of transductional fragments in heterogenotes obtained from diploid recipients. It appears likely that the exogenotic fragment does not lie freely in the cell but is closely coupled to the homologous genes in the chromosome, possibly in the same fashion as prophage. Further experiments are designed to test whether the exogenote is laterally synapsed or occupies a specific place in the linear linkage sequence.

4. 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 (A5); (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 trans heterogenotes 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.

5. Prophage relationships in lambda transduction. (E. M. Lederberg) The finding by Campbell and others (A6, A7) 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; 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.

6. DNA-mediated transduction: 'transformation'. Extensive trials of protoplasts as recipients of E. coli DNA have given no affirmative results, but should be pursued under a wider range of conditions. Some time was spent on developing a Hemophilus assay system as a control for the stability of DNA, absence of inhibitors, and so on in the E. coli trials. A culture which could grow in broth in the absence of serum was isolated and some effort was spent in the analysis of its nutrition and in the production of new auxotrophic mutants to serve as markers. However, the culture proved insusceptible to transduction, and although it resembled Hemophilus in the diagnostic feature of absolute dependence on hemin, it proved to be a contaminant. I was informed by the donor of the strain that this contaminant was already present in his stock culture.

Attempts to select mutants of an adenineless E. coli that could utilize external nucleotides and thereby have presumably developed a technique for their entry were not successful.

Despite these failures, all these investigations warrant continued, intensive study and will be given high priority during the next year. Dr. Kaiser's recent success in transducing Al markers to protoplasts with disrupted lambda is a hopeful lead.

7. Determination of sex. (Mr. Hirota and Mr. Richter). Maleness in wild type E. coli is determined by the presence of an F agent which is readily and contagiously transmitted from F^+ to F^- cells. Richter has analysed a number of non-infective, more fertile 'Hfr' mutants from the standpoint of their specific recombinational patterns, and their relationship to F^+. He concludes that Hfr 'mutation' consists of the transposition of a (cytoplasmic, v.i.) F agent to a chromosomal site, whence it is no longer readily transmissible. In the course of mating, it induces a break adjacent to its own locus, so that the Hfr mutant is very rarely recovered in sexual progeny. In some instances, the Hfr mutant may revert to an F^+ form, either the standard F^+ or one that has a high probability of regenerating the Hfr, giving a high incidence of oscillations: $Hfr \rightleftarrows F^+$.

Hirota (Ma) has found that F^+ cultures are massively converted by cobalt and acridine-orange to genetically stable F^-. Microculture experiments have excluded selection for spontaneous F^- mutants, and the loss of F must be directly induced by the dye. That, among various males, only infective F^+ cultures can be demasculinized is further evidence for the extranuclear location of F in these strains.

REFERENCES

(See complete bibliography appended for references to work from this laboratory.)

OTHER REFERENCES

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A5 - Kalckar, H. (1957). In "Symposium on the Chemical Basis of Heredity" (W. D. McElroy and B. Glass, eds.), pp. 463-467.

A6 - Campbell, A. (1957). "Transduction and segregation in Escherichia coli K12. Virology 4, 366-384.

A7 - Arber, W., Kellenberger, G., and Weigle, J. (1957). "La defectuosite du phage lambda transducteur. Schweizerische Zeitschrift fur Allgemeine Pathologie und Bakteriologie 20, 659-665.