RESEARCH PROPOSAL SUBMITTED TO THE OFFICE OF NAVAL RESEARCH

TITLE: *Engineering Analysis of a Genetic Switch*

INSTITUTION: *University of California*
Berkeley, CA 94720

PRINCIPAL INVESTIGATOR(S):

Principal Investigator	Adam Arkin	**Date**	5/30/00	**Co-Principal Investigator**	John Little	**Date**	5/30/00
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INSTITUTIONAL ENDORSEMENTS

University of California, Berkeley

Administrative Head		**Date**	00/00/00	**Institutional Financial Officer**		**Date**	00/00/00
Address:				**Address**:			
Phone:				**Phone**:			
Fax:				**Fax**:			

University of Arizona

Administrative Head		**Date**	00/00/00	**Institutional Financial Officer**		**Date**	00/00/00
Address:				**Address**:			
Phone:				**Phone**:			
Fax:				**Fax**:			

TOTAL COST: *Dollar amount requested (sum of direct plus indirect costs)*

PROPOSED START DATE: June 1, 2000

PROPOSED END DATE: May 31, 2003

DATE SUBMITTED: January 31, 2000

THIS PROPOSAL IS SUBMITTED PURSUANT TO THE ONR GUIDE TO PROGRAMS, BROAD AGENCY ANNOUNCEMENT, DATED 17 JULY 1997

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Statement of Objectives

We shall combine experimental and computational approaches to explore the systems behavior of a genetic switch in phage λ. The molecular components of this switch are well understood. Though structurally simple, this small regulatory network displays a threshold response with a controlled set-point. We shall develop molecularly detailed, experimentally validated models of this process in order to divine the engineering principles of this circuitry. We will then use this understanding to design and implement novel "induction" switches with chosen threshold steepness and set-points to demonstrate that these principles have indeed been understood and perhaps to be used for biotechnology purposes.

Statement of the Approach

A combination of genetics, site-directed mutagenesis, physiology and biochemistry will be used to probe the contributions of switch components to the threshold and set-point of the switch. Concurrently, our computational models of the λ lysis/lysogeny decision will be extended to include the host SOS response and the particular biochemistry and genetics of the switch. These models will be used to explain the complex dynamical phenotypes scored during the experiments, and to design experiments that test and validate each sub-part of the model. Thus, as in other engineering disciplines, a tight cycle will be created between theory and experiment. Finally, the model will be used as a computer-aided design tool for the production of switches with specified thresholds and set-points.

Statement of Significance

A detailed understanding of cellular events at the molecular level must include an accurate description of systems behavior. Currently, we do not have such a description for any system. Phage λ is one of the simplest biological organisms to exhibit interesting systems behavior. The wealth of quantitative information in this system, and its powerful genetics and biochemistry, make it ideally suited for producing and testing detailed models of systems behavior. In turn, such models are expected to provide general insights into systems behavior, allow design of engineered systems, and serve as a basis for designing interventions in cellular events, eventually putting molecular medicine on the same practical footing as other engineering disciplines.

Key Word/Phrases (5 items that are not represented in the title)

Molecular Bioengineering
Molecular Medicine
Genetic Network Analysis
Computational Biology
Genetic Engineering/Virology

Body of Proposal

Synopsis

Phage λ, which infects *Escherichia coli*, is a temperate phage. That is, after infecting a cell, λ can follow either of two pathways. It can grow lytically, producing more virions, or it can set up a stable association with the host, the "lysogenic" state. These states represent alternative patterns of gene expression. Moreover, the lysogenic state can be switched to the lytic state by induction of the host SOS response. Hence, λ is one of the simplest organisms to exhibit interesting gene regulatory behavior. Both host and virus have been fully sequenced and have been studied intensively and quantitatively for the past fifty years. However, even this relatively simple system exhibits system behaviors that have not yet been understood in sufficient detail to allow the robust prediction of the effect of mutation, pharmacological and environmental perturbation on the regulatory circuitry. The λ phage induction switch, mediated by the *E. coli* SOS stress response system, is an excellent model system for studying, in quantitative molecular detail, the cellular engineering principles by which the phage creates a threshold induction response to UV damage of the host. In this study, we propose to dissect this switch through the use of directed genetic engineering of the promoter structure, protein-protein and protein/DNA interaction strengths, and protein and mRNA stabilities. The phenotypes to be scored include the steepness of the threshold of phage induction as a function of UV exposure and the set-point of this threshold. These phenotypes are complex system properties of the induction genetic network thus the data generated from these experiments will be compared to prediction of a rigorous computer model of the underlying molecular mechanisms. This model will be constructed using models of the genetic apparatus previously constructed for prediction of the lysis/lysogeny decision that occurs earlier in the infection process. The model will be supplemented and augmented with data on the host SOS response and better parameters for the λ-specific chemistry than was available at the time of the previous model. The mismatch between the experimental results and the mathematical model will be used to refine the model into a predictive tools which will then been used as a platform for rationally design induction switches with chosen threshold steepness and set-point.

Introduction

It is a surprising statement in this era that even the simplest and best-studied biological systems exhibit behaviors that are difficult to understand from the qualitative pathway diagrams of their biochemical and genetic networks. However, as has been shown time and again, beautiful theories about system functions are often brought down by a single new experimental fact. Of course, the reverse is true as well; experimental facts often need to be reinterpreted in light of a new theory. In no other system are these maxims more elegantly demonstrated than in the study of the lysis/lysogeny and induction switches fundamental to the infection of *Escherichia coli* by the bacteriophage.

This phage has been the focus of intensive study for decades, and its patterns of gene regulation are well understood. When λ infects an *E. coli* cell, the infected cell can respond in either of two ways. First, it can follow a pattern of **lytic** growth, typical of many viruses, in which the virus expresses a set of early genes, replicates its DNA, expresses late genes, packages the DNA into mature virions, and lyses, releasing the virions. Alternatively, it can enter the **lysogenic** pathway. In this case, makes a regulatory protein called CI, which acts as a repressor to turn off the lytic genes, and it physically integrates its DNA into that of the host. In the descendants of the cell, expression of CI continues indefinitely, leading to continued repression of the lytic genes, and this lysogenic state is extremely stable. However, it is possible to switch the regulatory state of the cell from lysogenic to lytic, in a process called "prophage induction", by inducing the host SOS regulatory system. When this happens, CI is inactivated by proteolytic cleavage, expression of the lytic genes begins, CI expression is turned off, and the cell follows the lytic pathway. This change of state, often termed the "genetic switch", is the main focus of the present proposal.

The regulation of the *c*I gene is complex. λ *c*I is expressed from either of two different promoters, and these promoters are used in different phases of the λ life cycle. The initial lysis-lysogeny decision depends largely on whether *c*I is expressed from an establishment promoter called P_{RE}. Expression from P_{RE} depends in turn on the presence of a positive regulatory protein, CII. The level of CII in a cell depends

on the cell physiology in ways that are poorly understood. Once the lysogenic state is established, it is maintained by a second promoter, P_{RM}, whose expression is dependent on the presence of CI in the cell. A map showing the location of the relevant regulatory sites and genes is shown in Fig. 1.

Lambda has a second regulatory gene, *cro*, whose product acts in a way antagonistic to CI. If Cro is expressed, it turns off CI expression, favoring the lytic state. We may say that if Cro wins, it continues to win, while if CI wins, it continues to win. The situation is not entirely symmetrical, in that the lytic state need not be stable indefinitely.

These two regulatory proteins both act at a complex regulatory region termed the O_R region. This site lies between the *c*I and *cro* genes. It contains three binding sites, O_R1, O_R2 and O_R3, to which both CI and Cro bind. In wild type λ, they bind to these sites with differing affinities. CI binds tightly to O_R1 and weakly to sites 2 and 3; however, it also binds cooperatively, so that when O_R1 is occupied, O_R2 becomes occupied as well. Activation of P_{RM} requires binding of CI to O_R2, so that when these two sites are occupied, P_{RM} is stimulated. When CI is bound to these two sites, it also represses expression of *cro* from the P_R promoter (Fig. 1). Hence, the state of a cell containing CI and no Cro is perpetuated. Conversely, when Cro is present, it binds most tightly to O_R3, repressing P_{RM} without affecting its own expression from P_R. Again, the pattern of expression is perpetuated.

As stated, these occupancy patterns are seen in wild type λ. Indeed, for many years it was believed that they are crucial to the proper operation of the regulatory circuitry, and was cited as the prime example of the belief that "it's all in the details", that is, the exact details of the circuitry are crucial. However, this belief was never tested experimentally. We recently undertook such a test, and found, perhaps surprisingly, that the differential occupancy patterns are **not** required for the qualitatively proper operation of the switch (Little, Shepley et al. 1999). Three different variants were made in which the sites at the locations O_R1 and O_R3 are the same. Strikingly, these phages had qualitatively normal behavior, in that they could grow lytically, form highly stable lysogens, and these lysogens could undergo prophage induction. Hence, for these parameters, the genetic switch is highly robust, a system property that is of considerable importance for evolution. This finding also confirms the belief that intuition is an unreliable guide for predicting system properties; despite its great intuitive plausibility, the reigning model was incorrect.

Similarly, in theoretical and computational work, Arkin, Ross and McAdams have developed detailed mathematical and computational models of the phage and *Escherichia coli* pathways that govern the two λ-phage decisions (Arkin, Ross et al. 1998). They have shown that, in a physically rigorous model of the λ lysis/lysogeny decision circuit, the noise inherent in gene expression (McAdams and Arkin 1997) can lead to irreducible heterogeneity in this decision; nonetheless, the phage robustly chooses one route or the other. In addition, they tested a number of mechanistic hypotheses about the HflA/HflB mediated degradation of CII and protection of CII by CIII production. The model: 1) Better explained data about percent lysogeny as a function of average phage input than any other model and resolved a conflict with the prevailing wisdom, 2) emphasized the importance of the HflA/HflB proteolytic system as the basis for the lysis/lysogeny switch during the initial infection rather than the OR1-OR3 competitive binding region (this region is certainly more central to the induction process) 3) yielded a prediction for the most likely class of CII/CIII degradative function that was ultimately experimentally proven correct (in its basic mechanisms). The study again proved that qualitative analysis of even this very simple four-promoter, five-gene system was inadequate for predicting detailed system function and understanding its engineering principles.

This failure of qualitative analysis is rooted in the fact that complex systems often exhibit properties that are not readily predicted from the behavior of their parts. Examples of system properties are feedback, in which the output of a system serves as input at a later time, and robustness, or the resistance of a system to changes in its components. Particularly interesting systems are those, such as the system we propose to study here, that exhibit multiple alternative stable states. Such systems have additional system properties such as stability (resistance to change of state due to perturbations) and threshold behavior.

These "emergent" properties of a complex system arise due to factors such as non-linearity, feedback, and stochastic behavior. Because of these features, our intuition is a poor guide to predicting system behavior. In order to understand this behavior, mathematical analysis and experimental testing must be combined. Models are capable of predicting the effects of changes in system components on the behavior of the system. Such predictions can then be tested experimentally, providing a check on the model and allowing a dynamic interplay between model refinement and experimental validation. In this proposal, we shall use a tight cycle of experiment, theory and computation in order to elucidate the systems engineering of the prophage induction process in λ phage development.

The induction process was not a part of the original computer model, but is a crucial part of the λ regulatory circuitry. Prophage induction occurs when the SOS response, a cellular response to DNA damage, is activated. It exhibits two important aspects of system behavior: First, it shows threshold behavior (Fig. 2). Below a certain level of DNA damage, little or no induction occurs. At a certain threshold, induction abruptly becomes efficient, and most cells are induced. It is believed, though not proven experimentally, that this threshold arises because of cooperative DNA binding by CI to O_R1 and O_R2. Second, this threshold has a set-point. This set-point lies at about the level of DNA damage that begins to kill the cells under laboratory conditions. It is believed, again without proof, that this set-point has evolved so that lysogens will not induce at doses that the cells can survive. This property is often likened informally to rats deserting a sinking ship.

Hence, the process of prophage induction exhibits two related system properties: It has threshold behavior--that is, the curve describing the switch is steep over a narrow range of inducing doses—and the threshold has a particular set-point. The goal of this proposal is to understand why the curve is steep, and how the set-point is set at the particular value seen in wild-type. We propose to investigate these related aspects of system behavior.

This proposal, then, has four specific aims:

1. The creation of a detailed computer model of SOS-mediated prophage induction of λ and analysis of this model to predict the determinants of the threshold steepness and set-point position.
2. Use of the model to prototype genetic experiments to test these predictions.
3. Experimental implementation of genetic constructs designed to test these hypotheses and the computer prediction. Quantitative analysis of the behavior of the modified phage to compare to model predictions. Using this comparison to point to modification to the model in step 1.
4. Following validation of the wild-type model and mutant models, the use of these models as a platform for designing induction switches with specified threshold gradients and set points.

It is only by completing all four tasks that one proves complete engineering understanding of the switch circuitry. The study serves as a model for the experimental/computational cycle that will be necessary for understanding more complex circuitry and will provide an example of how to combine experimental and computational approaches to engineer specific genetic network function. Finally, this project serves as a step for producing a detailed model of the entire viral life-cycle.

Technical Approach

Both the Little and Arkin laboratories have experience working with λ-phage. John Little has over thirty years of experience with the experimental and quantitative aspects of λ-phage infection and twenty years of experience with the SOS system. Adam Arkin is an expert in modeling genetic and biochemical reaction networks and was a developer of the λ-phage lysis/lysogeny decision model.

Experimental

1. Analysis of prophage induction is straightforward. Cells are exposed to graded doses of UV irradiation, which damages DNA, and the resulting burst of phage is determined (see Fig. 2).

2. In the course of our work on robustness, we discovered that the "symmetrical" variants are more readily induced than is the wild-type (Fig. 2). This probably results in part because lysogens contain lower levels of CI. As a complication, recent evidence suggests that the promoter strengths of at least one of these variants has been altered as well, despite the fact that the changes do not affect the -35 and -10 regions of P_R or P_{RM}. In any case, these variants can be modeled as an initial test of the model for the genetic switch.

3. Starting from the most sensitive variant, O_R323, we were able to isolate variants that were more resistant to UV induction. Hence, these "hair-triggerless" variants have altered patterns of gene expression. These variants appear to be of several types:
    a. A few mutants lie in *c*I, and likely make the protein more difficult to cleave. A large body of evidence from our work (Little 1993) strongly suggests that CI contains a built-in self-cleavage activity, which is triggered in the cell by interaction with an activated form of RecA protein. Many mutations exist in *c*I that change the rate of this reaction, and the hair-triggerless variants in *c*I are presumably of this type. The single mutants (separated from O_R323) are difficult to induce.
    b. One mutation is probably an up-promoter in P_{RM}, making this promoter several-fold stronger. Presumably, this variant makes more CI. This P_{RM} mutation has not yet been separated from the altered O_R1 site in this phage.
    c. One mutation reverts the fifth position in the O_R3 site at O_R1 back to its identity in O_R1; this change represents a second example of evolution towards a wild-type state.

    This approach of isolating hair-triggerless variants will be extended with O_R323.

4. We propose also to start with wild-type lysogens and isolate variants that have altered set-points. Initial efforts to do this have revealed an interesting complication. λ can form "double lysogens", in which two prophages are present in tandem. We found that double lysogens are more resistant to UV induction. This is interesting in that it suggests that λ has a diversity of response to induction, in that its set-point is not simply that of a single lysogen but has several values. At the same time, it complicates genetic approaches to isolating hard-to-induce lysogens. We are developing strategies to obviate this complication.

5. Finally, it has long been believed that the threshold is sharp due to cooperative binding of CI to O_R1 and O_R2. We will test this model by reducing or eliminating cooperative binding. This will be done by mutation of *c*I in residues known to be required for cooperative binding. This will probably require increasing the binding of CI to O_R2, either by mutation of the O_R region, an increase in the strength of P_{RM}, or by mutation of *c*I in a way as to increase its affinity for its sites. In addition, we will take a classical genetic approach to isolating such mutants, once we know the phenotypes of site-directed changes.

Computer Modeling

All models for the λ induction process will be constructed in the Bio/Spice biological modeling package developed in the Arkin group. The package allows mixed ODE/stochastic simulation of prokaryotic cellular chemistry and can track cell division and population level dynamics. Currently, the validated λ lysis/lysogeny decision model is resident in this system. This means that the lion's share of the λ side of the wild-type induction model is already in existence. However, models of the *E. coli* SOS response (in more sophistication than simply the inclusion of RecA-mediated cleavage of CI monomers) need to be developed. In addition, models of all the mutant strains of λ described above need to be constructed and

validated against the data from the Little laboratory. Each of these models will be analyzed for the dynamical bifurcation structure in order to predict the parameters most important for determining threshold steepness and set-point. The dependency on prophage number (1 or 2) will be tested. Because of the complexity of the model, it is likely we will have to resort to numerical continuation to obtain these parameters. Simulations of these models will then be used to predict total experimental outcome.

Finally, the Bio/Spice tools will be used in its design mode to come up with genetic circuits designed to produce particular threshold/set-point behaviors. This relies on having the validated wild-type and mutant models from which pieces may be rearranged to produce the desired results.

It should be noted, however, that Bio/Spice is far from a finished product. It is currently a tool under development and the rigorous and correct modeling of biological systems is still a field of intensive research. There are a number of difficult challenges in creating accurate models of cellular systems. The first is the collection of quantitative kinetic, thermodynamic and mechanistic data necessary for creating dynamical models of the piece parts of the system. The second is the collection of data on the system properties of the process of interest in sufficient detail to allow comparison to model predictions. The third is the rigorous testing of model predictive power through the detailed measurement of the perturbed or mutated system. In electronic and mechanical system these three tasks are achieved through very tight association of the experimental and computational scientists. This association is still rare in biology. Thus, the development of good "engineering" software for biology has been strongly hampered. In this work, not only do we propose to cycle through all three tasks with a model system that allows fine experimental manipulation but we have an ideal team to rapidly test the efficacy of this cycle in biology.

Schedule

We anticipate a three year time-line for completion of all tasks:

Year 1: Construction of wild-type λ-phage induction model and SOS response will be completed and preliminary experimental validations will occur (measurement of protein and gene expression, characterization of dose-response curves). Initial models of all mutants from (Little, Shepley et al. 1999) will be built and tested against results from that paper. Initial models testing the role of operator order and affinity, cooperative CI binding, etc. on system properties will be made. Strains of λ necessary for testing these predictions will be made.

Year 2: Quantitative measurement of different strain dynamics following gene and protein expression and graded UV dose-response. Collation of data into database and model testing. Revision of model based on results, development of specific genetic tests of new model.

Year 3: With validated wild-type and mutant models, λ phage with specified threshold and set-point responses will be designed and implemented.

Summary

All of these tasks represent relatively straightforward low-risk subtasks. However, the unique extremely tight coupling of experiment and theory makes this project innovative. Moreover, the elucidation of an important class of genetic switching mechanisms will allow a finer understanding of genetic control of developmental and infective processes. In addition this project will push us closer to a complete and detail, experimentally validated molecular-level model of a viral life cycle.

References

Arkin, A., J. Ross, et al. (1998). "Stochastic kinetic analysis of developmental pathway bifurcation in phage λ-infected *E. coli* cells." Genetics **149**(4): 1633-1648.
Little, J. W. (1993). "LexA cleavage and other self-processing reactions." Journal of Bacteriology **175**: 4943-4950.
Little, J. W., D. P. Shepley, et al. (1999). "Robustness of a gene regulatory circuit." EMBO J **18**(15): 4299-307.
McAdams, H. and A. Arkin (1997). "Stochastic Mechanisms in Gene Expression." Proceedings of the National Academy of Sciences, USA **94**: 814-819.

Recombinant DNA

Many of the experiments described will involve the use of recombinant DNA. This use is in compliance with DHHS recombinant DNA regulations. The University of Arizona, where the experimental work will be carried out, has an Institutional Biosafety Committee, and approval of this committee will be obtained.

Facilities available, especially unique facilities or capabilities

University of California, Berkeley

Computational Facilities

In addition to a cluster of NT and Unix workstations and development software for development of the biological models, the Arkin group is one of the recipient of an IBM SUR grant that has provided a four node (8 processor) IBM SP parallel processing computer ideal for the execution of the Bio/SPICE simulation kernel. In addition, a set of newly created biological network and modeling databases are available on these computer systems. There is also access to the Lawrence Berkeley National Laboratory's NERSC supercomputer center for more intense model sensitivity analyses and testing.

Laboratory Facilities

Full molecular biological laboratory equipment and facilities are available at the same site as the computational core. This Arkin group has access and space in these facilitates suitable for any work necessary to aid in this collaboration and so that visiting biologists may perform work on site next to the computational chemists and biologists.

Office Facilities

Every post-doctoral, graduate student and programmer has his or her own desk, computer equipment and materials. In addition, some of these have their own laboratory benches. There is room sufficient to expand by one or two visitors during the year.

University of Arizona

Laboratory Facilities

The Little laboratory is well equipped for and highly experienced at standard recombinant DNA, bacteriological, and biochemical work. The building has core equipment rooms and a dishwashing facility. The Division of Biotechnology, located in the same building, routinely does DNA sequencing.

Office Facilities

The PI has an office and there is an additional office for housing visitors. In the lab, each investigator has his/her own bench and desk.

Annual Budget (indicate institutional matching where appropriate)

University of California, Berkeley

Personnel salaries, wages and fringe benefits (indicate % effort)

A. Salaries and Wages	**Effort**	**Base**	**Est. Cost**	**Total Cost**
A.1 PI: Adam Arkin	0.05	105,000	5,290	
A.2 Post-Doc	0.75	36,000	27,000	
Total Labor	0.80			32,290

B. Fringe Benefits	**Rate**	**Base**	**Est Cost**	**Total Cost**
B.1 PI: Adam Arkin	0.092	5,290	487	
B.2 Post-Doc	0.170	27,000	4590	
Total Benefits				5,077

C. Total Salaries and Fringe Benefits	37,367

D. Scientific and Support Burden				
D.1 Scientific Burden (on C)	0.0	0	0	
Total Burden				0

Equipment purchase and maintenance

F. Purchases		
F.1 Equipment	$5,000	
F.2 Other procurements; lab & office supplies	$2,500	
F.4 Procurement burden and Material Handling	720	
Total Purchases		8,220

Materials and supplies (itemize major categories)

H. Other Direct Costs—Overheaded		
H.1 Publications	1,100	
H.2 Central Computing Facilities	1,000	
H.3 Recharges	0	
H.4 Miscellaneous expenses	0	
Total		2,200

I. Other Direct Costs- No Overhead	
I.1 Conferences and Workshops	0
I.2 Stipends	0
I.3 Electricity	0
Total	850

Travel

G. Travel	
G.1 Domestic	2,500
G.2 Foreign	0
Total Travel	2,500

Totals (provide annual breakdown and cumulative summary)

J. Total Direct Costs and Burdens	46,060	
L. Total Laboratory Costs	5,077	
M. **Total Costs**		51,137

University of Arizona

Personnel salaries, wages and fringe benefits (indicate % effort)

Salaries and Wages	**Effort**	**Base**	**Est. Cost**	**Total Cost**
PI: John Little	0.33	72,000	24,000	
Res. Spec. Sr., Robin Roberts	1.0	35,000	35,000	
Total Salaries	1.25			59,000

Fringe Benefits	**Rate**	**Base**	**Est Cost**	**Total Cost**
PI: John Little	0.182	24,000	4,368	
Res. Spec. Sr.	0.224	35,000	7,840	
Total Benefits				12,208

Total Salaries and Fringe Benefits 71,208

Equipment purchase and maintenance

Misc. small equipment	2,000	
Service contracts	1,000	
Total		3,000

Materials and supplies (itemize major categories)

Biochemical supplies, growth media	7,000	
Oligonucleotide synthesis	5,000	
DNA sequencing	5,000	
Glassware	2,000	
Total materials and supplies		19,000

Other Direct Costs

Publications	1,500	
Office supplies, telephone, copying	750	
Journals, books	500	
Total		2,750

Travel

Domestic	2,500	
Foreign	0	
Total Travel		2,500

Total direct costs 98,458

Indirect Costs

	Rate	**Base**	**Est Cost**	**Total Cost**
	0.515	98,458	50,705.9	
Total				50,705.9

Totals (provide annual breakdown and cumulative summary)

Total Direct Costs	98,458	
Indirect Costs	50,706	
Total Costs		**149,164**
Three-year total (including 4% increment per year for 2nd and 3rd years)		**465,630**

Budget Justifications

The salary of the PI (Little) is an academic year salary. The University of Arizona allows this salary to be supplemented by up to 1/3 as a summer salary; the amount requested is the entire 1/3.

Supplies requested are required to carry out the microbiological, biochemical, and recombinant DNA work entailed in the project.

Travel funds are requested for two purposes: First, frequent travel between Tucson and Berkeley, the two performance sites, in order to foster interchange between the two groups; and second, for the PI to attend a national meeting.

CV's for investigators and consultants

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Adam P. Arkin, Ph.D.	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Carleton College, MN	B.A.	1988	Chemistry
Massachusetts Institute of Technology, MA	Ph.D.	1992	Physical Chemistry
Stanford University (Chemistry), CA	Postdoc	1992-95	Nonlinear Chem. Systems
Stanford University (Developmental Biology), CA	Postdoc	1995-1997	Modeling Development

Professional Experience

July 1999- Present	Assistant Professor, Departments of Bioengineering and Chemistry, University of California, Berkeley
	Faculty Scientist, Computational and Theoretical Biology Department, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA
January 1998-July 1999	Staff Scientist, Computational and Theoretical Biology Department, Physical Biosciences Division, E.O. Lawrence Berkeley National Laboratory, University of California, Berkeley, CA

Address

1 Cyclotron Road, MS 3-144, Berkeley, California, 94720

Honors

M.I.T. Technology Review Top 100 Young Innovator Award Recipient.

Teaching

Chemistry 130A: Biophysical Chemistry

Selected Publications

Refereed Journals

1. **Arkin, A.P.**, Youvan D.C. (1992) An Algorithm for Protein Engineering: Simulations of Recursive Ensemble Mutagenesis. *Proc. Natl. Acad. Sci. USA* **89**(16):7811-7815.
2. **Arkin, A.P**., Ross, J. (1994) Computational Functions in Biochemical Reaction Networks. *Biophysical Journal*. **67**:560-578.
3. **Arkin, A.P.**, Ross, J. (1995) Statistical Construction of Chemical Reaction Mechanisms from Measured Time-Series. *J. Phys. Chem.* **99**: 970-979.
4. McAdams, H., **Arkin. A.P.** (1997) Stochastic Mechanisms in Gene Expression. *Proc. Natl. Acad. Sci., USA* . **94**(3):814.
5. Swanson, C., **Arkin, A.P.**, Ross, J. (1997) An Endogenous Calcium Oscillator May Control Early Embryonic Division. *Proc. Natl. Acad. Sci., USA* . **94**(4):1194.

6. **Arkin, A.P** ,.Shen, P.-D., Ross, J. (1997) A Test Case of Correlation Metric Construction of a Reaction Pathways from Measurements. *Science*. **277**(5330): 1275.
7. McAdams, H. H., **Arkin, A.P.** (1998) Simulation of Prokaryotic Genetic Networks. *Annu. Rev. Biophys. Biomol. Struct.* **27**: 199-244
8. **Arkin, A.P**., Ross, J., McAdams, H.H. (1998) Stochastic Kinetic Analysis of a Developmental Pathway Bifurcation in Phage-λ *Escherichia coli*. *Genetics.* **149**(4):1633-1648.
9. McAdams, H.H., **Arkin, A.P** (1999) Genetic Regulation at the Nanomolar Scale: It's a Noisy Business! *TIGS*. **15**(2): 65-69.
10. **Arkin, A.P.** (1999) Signal Processing by Biochemical Reaction Networks. In: Biodynamics. J. Walleczek, ed. Cambridge University Press, Cambridge.In Press.

Other Significant Publications

1. with Gary Stix. (1991) Protein Probe: Remote Sensing Technique Screens Bacterial Colonies. *Scientific American*. May issue. p. 123.
2. **Arkin, A.P.**, Youvan, D.C. (1992) Digital Imaging Spectroscopy. In: The Photosynthetic Reaction Center J. Deisenhofer & J.R. Norris eds. 133-154.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
John W. Little, Ph.D.	Professor of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Stanford University, Stanford, CA	B.S.	1962	Chemistry
Stanford University, Stanford, CA	Ph.D.	1967	Biochemistry

Professional Experience:

Senior Assistant Scientist, U.S. Public Health Service, 1967-1969
Senior Staff Fellow, National Institutes of Health, Bethesda, MD 1969-1972
Post-doctoral Fellow, Laboratory of P.C. Hanawalt, Department of Biological Sciences, Stanford University, 1973-1976
Biochemist, Stanford Research Institute, Menlo Park, CA, 1/77-8/77
Research Associate, Department of Microbiology, University of Arizona, 8/77-6/78
Adjunct Assistant Professor, Department of Microbiology, University of Arizona, 7/78-6/80
Adjunct Associate Professor, Department of Medical and Molecular Microbiology, University of Arizona, 7/80-12/81
Assistant Professor of Biochemistry, University of Arizona, 1/82-7/85
Assistant Professor of Molecular and Cellular Biology (joint appointment), Univ. of Arizona, 2/84-7/85
Associate Professor of Biochemistry, University of Arizona, 8/85-7/91
Associate Professor of Molecular and Cellular Biology, University of Arizona, 8/85-7/91
Professor of Biochemistry, University of Arizona, 8/91-present
Professor of Molecular and Cellular Biology, University of Arizona, 8/91-present

Honors and Awards:

National Merit Scholar, Stanford University, 1958-1962
Phi Beta Kappa, Stanford University, 1962
Graduation with Great Distinction, Stanford University, 1962
US Public Health Service Predoctoral Fellowship, Dept. of Biochemistry, Stanford, 1962-1966

Bibliography

Representative Publications

Little, J.W., Edmiston, S.H., Pacelli, L.Z. and Mount, D.W. (1980) Cleavage of the *lexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. **77**: 3225-3229.

Little, J.W., Mount, D.W. and Yanisch-Perron, C. (1981) Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. **78**: 4199-4203.

Little, J.W. and Mount, D.W. (1982) The SOS regulatory system of *E. coli*. Cell **29**: 11-22.

Little, J.W. (1984) Autodigestion of lexA and phage lambda repressors. Proc. Natl. Acad. Sci. **81**: 1375-1379.

Slilaty, S.N. and Little, J.W. (1987) Lysine-156 and serine-119 are required for *lexA* repressor cleavage: A possible mechanism. Proc. Natl. Acad. Sci. USA **84**: 3987-3991.

Lin, L. and Little, J.W. (1989) Autodigestion and RecA-dependent Cleavage of Ind^- Mutant LexA Proteins. J. Mol. Biol. **210**: 439-452.

Smith, M.H., Cavenagh, M.M. and Little, J.W. (1991). Mutant LexA proteins with an increased rate of *in vivo* cleavage. Proc. Natl. Acad. Sci. USA **88**: 7356-7360.

Kim, B. and Little, J.W. (1992). Dimerization of a specific DNA-binding protein on the DNA. Science **255**: 203-206.

Roland, K.L, Smith, M.H., Rupley, J.A. and Little, J.W. (1992). *In vitro* analysis of mutant LexA proteins with an increased rate of cleavage. J. Mol. Biol. **228**: 395-408.

Carlson, N.G. and Little, J.W. (1993). Highly cooperative DNA binding by the coliphage HK022 repressor. J. Mol. Biol. **230**: 1108-1130.

Kim, B. and Little, J.W. (1993). LexA and λ CI repressors as enzymes: Specific cleavage in an intermolecular reaction. Cell **73**: 1165-1173.

Little, J.W. (1993). LexA cleavage and other self-processing reactions. J. Bacteriol. **175**: 4943-4950.

Ennis, D.G., Little, J.W. and Mount, D.W. (1993). Novel mechanism for UV sensitivity and apparent UV nonmutability of *recA432* mutants: Persistent LexA cleavage following SOS induction. J. Bacteriol. **175**: 7373-7382.

Carlson, N.G. and Little, J.W. (1993). A novel anti-virulence element in the temperate bacteriophage HK022. J. Bacteriol. **175**: 7541-7549.

Mao, C., Carlson, N.G. and Little, J.W. (1994). Cooperative DNA-protein interactions: Effects of changing the spacing between adjacent binding sites. J. Mol. Biol. **235**: 532-544.

Shepley, D. and Little, J.W. (1996). Mutant LexA proteins with specific defects in autodigestion. Proc. Natl. Acad. Sci. USA **93**: 11528-11533 (1996).

Liu, Z. and Little, J.W. (1998). Inter-site spacing controls the mode of cooperative DNA-protein interactions: Implications for evolution of regulatory circuitry. J.Mol. Biol. **278**: 331-338.

Mao, C. and Little, J.W. (1998). Mutants affecting cooperative DNA binding of HK022 CI repressor. J. Mol. Biol. **279**: 31-48.

Little, J.W., Shepley, D.P. and Wert, D.W. (1999) Robustness of a gene regulatory circuit. EMBO J. **18**: 4299-4307.

OTHER SUPPORT

J. W. Little

"RO1 GM24178-21 07/01/96-06/30/00 33%
NIH/NIGMS $148,402
"Gene Regulation in Phages Lambda and HK022"

OVERLAP

This grant will end on 6/30/00. I intend to apply for a renewal to continue these studies. The projects in this renewal have a close relationship to the work proposed in the present proposal, in the sense that they deal with similar issues and are addressed in similar ways, using similar tools. I believe, however, that the two projects will be synergistic rather than overlapping. Tools and mutants generated in each will be useful for the other. One direct area of overlap that I anticipate is that the NIH renewal will probably include studies of the genetic switch like those proposed here. How we will deal with this depends on the timing of funding. If the present proposal is funded before the NIH renewal is submitted, I will remove studies about the genetic switch from the NIH renewal. If the present proposal is funded while the NIH renewal is under review, I will inform NIH that this specific aim is to be deleted, and request that the funds be reallocated towards the other specific aims of the NIH proposal. If both proposals are funded, we intend to split the efforts of the PI and the Research Specialist Senior equally between the two grants, and to use the funds thereby made available in the present budget to hire a post-doctoral fellow.

Recombinant DNA

Many of the experiments described will involve the use of recombinant DNA. This use is in compliance with DHHS recombinant DNA regulations. The University of Arizona, where the experimental work will be carried out, has an Institutional Biosafety Committee, and approval of this committee will be obtained.

For all current and pending support (including proposals under review) of each investigator list:

1. Project title and summary

2. Source and amount of funding

(annual direct costs; provide grant numbers for current grants)

3. Percentage effort devoted to each project

4. State how projects are related to proposed effort and indicate degree of overlap

List other agencies to which this proposal has been or will be submitted

Special information regarding certain types of experiments

1. Experiments involving animals require submission of a DOD approved protocol (call 703-696-4760 for a copy)
2. Experiments involving human subjects require: a) Statement of compliance with DHHS regulations, "Protection of Human Subjects" (32 Code of Federal Regulations, Part 219, 1 July 1992); b) Institutional Review Board Form
3. Experiments involving recombinant DNA require a statement of compliance with DHHS rDNA regulations

Certifications

These are required per OMB Circular A-129, Executive Order 12549, PL 100-690, and, for proposals exceeding $100,000, Section 1352, Title 31, U.S.C. (PL 101-121, Section 319). See your institutional contracts office for certification forms or call 703-696-4509 (1 copy only)

Appendices

Preliminary data, manuscripts, reprints, and any other supporting materials (2 copies only)