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SUMMARY STATEMENT
(Privileged Communication)

Release Date: 05/12/2014

Application Number: 1 DP2 OD018950-01

Principal Investigator

KOSURI, SRIRAM SCD

Applicant Organization: UNIVERSITY OF CALIFORNIA LOS ANGELES

Review Group: ZRG1 MOSS-C (56)
Center for Scientific Review Special Emphasis Panel
RFA RM13-007: New Innovator Award

Meeting Date: 04/24/2014
Council: MAY 2014
Requested Start: 09/30/2014

RFA/PA: RM13-007
PCC: 2HR200

Dual IC(s): RM

Project Title: REVERSE GENOMICS OF REGULATORY ELEMENTS GOVERNING SPLICING

SRG Action: Impact Score: 22

Next Steps: Visit http://grants.nih.gov/grants/next_steps.htm

Human Subjects: 10-No human subjects involved

Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year	Direct Costs Requested	Estimated Total Cost
1	1,500,000	1,500,000
TOTAL	1,500,000	1,500,000

ADMINISTRATIVE NOTE: Since the NIH Director's New Innovator Award applications are reviewed differently from other NIH grant mechanisms, criterion scores and percentiles are not assigned. The budget shown above is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

EARLY STAGE INVESTIGATOR, NEW INVESTIGATOR

1DP2OD018950-01 Kosuri, Sriram

**EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR**

RESUME AND SUMMARY OF DISCUSSION: The goal of this new innovator application is to use high throughput reverse genomics experiments in determining cis-elements that control pre-mRNA splicing. This research poses fundamental biological questions that have the potential to provide systematic evidence about what genetic mutations do or do not alter function and how this process occurs. The review panel emphasized the significance of the proposed research in defining splice sites in mammalian cells, praised the innovative and clever approach, and viewed the candidate as a uniquely qualified, exceptionally trained investigator suitable to undertake this challenging project. Minor weaknesses in technical details of the application were noted, namely lack of information about constructs being used and weak proof-of-concept data demonstrating accuracy of approach. However, other reviewers argued that the principal investigator has unique training in a similar approach using bacteria and has demonstrated the functional feasibility of the assay, thereby increasing the likelihood of the research success. The panel concluded that the strengths of this highly innovative application outweigh the minor concerns and that the developed approach is likely to have high impact on our understanding of mechanisms of pathological mutagenesis, and molecular biology in general.

DESCRIPTION (provided by applicant): Differences in our individual genomes give rise to most of human diversity. A decade removed from the Human Genome Project, much of how the genome directs phenotype still remains a mystery. Consortia like ENCODE seek to identify functional DNA elements in humans and other model organisms by correlating functional outputs with sequence using genome-wide data sets. However, these studies do not necessarily improve our ability to interpret how DNA elements act in new contexts or when mutated. Such an understanding will be critical to predict the effects of sequence alterations on phenotype and to engineer biology for future medicinal or technological purposes. Combinations of DNA elements act as codes controlling particular functions like transcription, splicing, localization, and silencing. Deciphering these codes is difficult, as the limited set of natural variants is typically insufficient to control for variables such as sequence composition or element combinations. Proving that particular sequences have causative effects on gene expression requires carefully controlled reverse genetic studies. Conducting such experiments on genome-wide scales is difficult because of our inability to (1) rapidly alter the sequence and context of individual genetic elements and (2) quantify the consequences of thousands of such changes. My central vision is to decipher cis-regulatory codes controlling gene expression by scaling reverse genetics experiments to genomic scales using multiplexed measurements of defined synthetic DNA libraries. I will build upon my work developing next-generation gene synthesis technologies and multiplexed reporter assays to systematically determine how sequences governing mammalian gene expression act in concert by doing thousands of controlled experimental tests simultaneously. Here, we will apply these technological developments to study how genetic regulatory elements control the process of pre-mRNA splicing. The major sequence elements controlling splicing, namely the splice donor, acceptors, and branch sites, do not convey enough information to specify exon inclusion or exclusion alone. Other regulatory elements, such as exonic or intronic splicing enhancers and suppressors, are known to affect splicing in a complex code that can vary based on tissue or cell type. We will systematically interrogate and refine the splicing code by leveraging the new technological developments proposed here. Studying splicing will help focus development of a complete suite of tools and technologies, which will later let us attack other forms of cis-elements controlling gene regulation.

PUBLIC HEALTH RELEVANCE: Any individual's human genome will have several million deviations from the consensus human genome sequence. Understanding whether these mutations are relevant or important is difficult because most mutations are uncommon and we do not have perfect understanding of how sequence affects function. Here we will develop new high throughput methodologies to

understand how and the extent to which this genetic variation affects function to develop better genetic diagnostics and therapeutic interventions.

CRITIQUES

EDITORS' CRITIQUES

Critique 1: This is an outstanding, imaginative and high-impact proposal from a pioneer in synthetic biology and DNA-synthesis methods. The proposal is to define cis-acting regulatory sequences required for RNA splicing in mammalian cells by high-throughput oligo-gene synthesis, to create the variable splice sites, on a spliceable green/red-fluorescent reporter construct the color of which indicates splice efficiency. These genes are then integrated and the slice-competent and -deficient are sorted by color, sequence and the functional and non-functional sites identified. The question is a good one, (what are the sequence determinants of splicing in mammalian cells?), and the methods are outstanding and will be generally useful for many purposes. They will help mammalian cell biology approach the level of tractability now possible in bacterial systems. The applicant is a successful pioneer, and eminently likely to make a high impact statement with this award.

Biohazards:

- Acceptable

Critique 2: This is an ambitious project to better identify cis-acting regulatory elements (CREs) in bacteria as a first step of understanding regulation of splicing of mRNA in mammalian systems. While the principal investigator has extensive experience in manipulating and measuring CRE activity in bacterial systems, applying these strategies to cultured mammalian cells will be much more complicated in terms of multiplexing measurements, identifying long CREs and generating larger reporter libraries. Strategies to overcome these problems are only briefly described and little information on their validity is provided in the proposal. The technical details of genomic engineering proposed here are quite impressive, but some of these details require a substantial leap of faith about the accuracy of these measures. The ultimate goal is to gain a better understanding of the "splicing code" used to process pre-mRNA in mammalian cells. This proposal is extremely ambitious and technically sophisticated, but it is somewhat short on confirmatory data to validate some of the methods described here.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Biohazards:

Not Applicable (No Biohazards)

Select Agents:

Not Applicable (No Select Agents)

Budget and Period of Support:

Recommend as Requested

Critique 3: The proposal seeks to identify cis-regulatory elements involved in pre-mRNA splicing by constructing a splice-detection construct library (using a fluorescence read-out) that will be transfected into cells and sorted and sequenced to detect cis elements regulating splicing. The issue of extrapolating from these cloned constructs, to endogenous splicing where secondary structure and perhaps other modifications may impact, somewhat dampens enthusiasm for this proposal.

Critique 4: This new investigator has trained with pioneers of synthetic biology Arkin, Endy, and Church. He is recently appointed Assistant Professor at the University of California – Los Angeles in Chemistry and Biochemistry with excellent core support and generous set-up and colleagues in synthetic biology and splicing, among other relevant research areas. As a postdoc in the Church lab, Dr. Kosuri explored cis- regulatory elements upstream of a green fluorescent protein (GFP) reporter using a library of 27000 synthetic fusions screened for DNA and RNA. This proposal will apply synthetic chip-assembled DNAs, but will tackle the more complex problem of identifying splicing CRE and so must be adapted to longer sequences expressed ultimately in eukaryotic cells. This is a clever strategy in which splicing-dependent expression of GFP is normalized to red fluorescent (RFP) levels to identify cis-acting sequences which affect splicing. One limitation is that it may be difficult to assign effects to primary sequences versus RNA structure or proteins. Computational algorithms for deconvoluting the results were not described in detail, nor were any plans for translating the results into clinical applications. It is well known that splicing affects many processes and is linked to heritable disorders affecting multiple genes. Thus, this work is potentially of high impact. Although this is a logical extension of work done in the Church lab, the project is highly challenging, and this investigator is uniquely qualified to pursue the problem.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MEETING ROSTER

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RFA RM13-007: New Innovator Award
ZRG1 MOSS-C (56) R
April 24, 2014 - April 25, 2014

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