

PROGRAM CONTACT:
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SUMMARY STATEMENT
(Privileged Communication)

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Application Number: 1 F31 AI133950-01

SCHWARTZ,SAMANTHA
Emory University

Review Group: ZRG1 F13-C (20)
Center for Scientific Review Special Emphasis Panel
Fellowship: Infectious Diseases and Microbiology
Meeting Date: 03/16/2017
Council: MAY 2017 *PCC:* I5A
Requested Start: 07/01/2017

Project Title: Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by dsRNA
Requested: 3 Years

Sponsor: Conn, Graeme L
Department: GRS: GDBBS BCDB
Organization: EMORY UNIVERSITY
City, State: ATLANTA GEORGIA

SRG Action: Impact Score:17
Next Steps: Visit https://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.

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1F31AI133950-01 Schwartz, Samantha

RESUME AND SUMMARY OF DISCUSSION: An excellent predoctoral applicant, in this application, seeks training in virus – host interactions with a project that focuses on studying the regulation of the 2'-5'-oligoadenylate synthetase (OAS) by cytosolic double-stranded RNA (dsRNA). The applicant is a first-generation college student who achieved a very good undergraduate academic record and gained undergraduate research experience that resulted in one first-author and several co-author publications. She is now a graduate student in the Biochemistry, Cell & Developmental Biology (BCDB) program at Emory. She is viewed as very strong. The sponsor and co-sponsor are reviewed as very strong with complementary research expertise and experience in mentoring graduate students. The research training plan is well articulated, although some review it as somewhat risky (high risk, high reward). The applicant will need to learn new techniques such as x-ray crystallography and mass spectrometry. Some reviewers view this as a potential weakness, whereas others view it as a strength of an overall excellent training plan. The institutional environment is excellent. Overall, there is high enthusiasm for the applicant, outstanding sponsors, excellent institutional environment, and important potential impact on advancing our understanding of host-pathogen interactions.

DESCRIPTION (provided by applicant): The innate immune system is a broad set of critical intracellular and extracellular processes that limit viral infectivity. In order to provide its essential first line of defenses against pathogens, the innate immune system must be able to accurately distinguish “self” from foreign molecules. Misregulation of the innate immune system can cause increased persistence and susceptibility to viral infection and human diseases, such as interferonopathies. The 2'-5'-oligoadenylate synthetase (OAS) family of enzymes are important innate immune sensors of cytosolic double-stranded RNA (dsRNA). Attesting to the importance of the OAS/RNase L pathway, viruses have developed ways to evade OAS. Previous structural studies have revealed that dsRNA binding allosterically induces structural changes in OAS1 that reorganize the catalytic site to drive synthesis of 2'-5'- oligoadenylates from ATP. These 2'-5'-oligoadenylate secondary messengers activate a single known target, the latent ribonuclease (RNase L). Active RNase L in turn degrades viral and cellular RNA to halt viral replication. Although X-ray crystal structures have given some insight into how OAS1 is activated by dsRNA, we still understand very little about how specific features of the dsRNA contribute to the level of OAS1 activation. To address which specific features of dsRNA are required for potent OAS1 activation, we designed dsRNA hairpin variants, based on the RNA duplex used in the structural studies. Remarkably, while a single point mutation on one strand resulted in complete loss of OAS1 activity, the equivalent mutation on the opposite strand led to increased OAS1 activity. Despite these stark differences in ability to activate OAS1, both variants appear to bind OAS1 with similar affinity. Given these preliminary findings, I hypothesize that dsRNAs may contain competing OAS1 binding sites with remarkably different capacities to activate the protein in a context dependent manner. However, the molecular signatures defining these sites as activating and non-activating are unknown. The goal of this project is to determine how specific sequences in dsRNA, and their context, control regulation of OAS1 in the following two Specific Aims. Aim 1. To use complementary assays of OAS1 activity in vitro and in human cells to determine the features of dsRNA that lead to potent activation of OAS1. Aim 2. To use biochemical, biophysical, and structural approaches to define the molecular mechanism(s) by which the dsRNA hairpin variants differ in their effects upon OAS1 activation. These experiments will reveal new insights into the regulation of OAS1 by dsRNA. In doing so, I will enhance our understanding of host-pathogen interactions, such as how viruses might circumvent the OAS1/RNase L pathway by masking activating motifs to evade detection. My results will furthermore provide new insights into cellular translational control in the context of infection and potentially strengthen the foundations necessary to design effective treatments for viral infection.

PUBLIC HEALTH RELEVANCE: The innate immune system is our cell's front line defense against infecting pathogens. This project will investigate how one important RNA-sensing component of the innate immune system is regulated by specific molecular signatures within double-stranded RNA

molecules. Such studies are essential to understand how the innate immune system is controlled, how its effects can be circumvented by infecting viruses, and as a potential platform to design effective antiviral treatments.

CRITIQUE 1

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 1

Research Training Plan: 1

Training Potential: 1

Institutional Environment & Commitment to Training: 1

Overall Impact: Samantha (Sam) Schwartz is a student in the Biochemistry, Cell & Developmental Biology (BCDB) Graduate Program at Emory University. Dr. Graeme L. Conn (Associate Prof., Department of Biochemistry) and Dr. Anice Lowen (Assistant Prof., Department of Microbiology & Immunology) will serve as co-mentors. Dr. Conn has many years of mentoring experience and a well-established record in the area of viral non-coding RNAs and their interactions with innate immune proteins, PKR and OAS1. Dr. Lowen will provide career guidance as well as outstanding expertise in molecular virology and the cell culture systems. Together they provide an outstanding team for the proposed biochemical and virology research. The research training plan is well written with a central hypothesis and path to test the hypothesis. Moreover, the outcomes, pitfalls have been presented and while early in the research provide a vision of the road ahead. The training potential is very high based on the carefully articulated plan for professional development and research described by the applicant, sponsors and the institutional environment. Outstanding resources are available for the proposed research; and an extremely well detailed overview of all areas of training over the course of graduate school were provided; really a truly wonderful and carefully crafted description. Everything seems to be in place for solid technical, scientific and professional training and career development.

1. Fellowship Applicant:

Strengths

- Samantha (Sam) Schwartz is a student in the Biochemistry, Cell & Developmental Biology (BCDB) Graduate Program at Emory University.
- Excellent preliminary data.

Weaknesses

- None noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- Dr. Graeme L. Conn (Associate Prof., Department of Biochemistry) and Dr. Anice Lowen (Assistant Prof., Department of Microbiology & Immunology) will serve as co-mentors.
- Dr. Conn has many years of mentoring experience and a well-established record in the area of viral non-coding RNAs and their interactions with innate immune proteins, PKR and OAS1.
- Dr. Lowen will provide career guidance as well as outstanding expertise in molecular virology and the cell culture systems.

Weaknesses

- None noted.

3. Research Training Plan:

Strengths

- The research training plan is well written with a central hypothesis and path to test the hypothesis. Moreover, the outcomes, pitfalls have been presented and while early in the research provide a vision of the road ahead.

Weaknesses

- None noted.

4. Training Potential:

Strengths

- The training potential is very high based on the carefully articulated plan for professional development and research described by the applicant, sponsors and the institutional environment.
- Everything seems to be in place for solid technical, scientific and professional training and career development.

Weaknesses

- None noted.

5. Institutional Environment & Commitment to Training:

Strengths

- Outstanding resources are available for the proposed research; and an extremely well detailed overview of all areas of training over the course of graduate school were provided; really a truly wonderful and carefully crafted description. Everything seems to be in place for solid training.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Training in the Responsible Conduct of Research:

Comments on Format (Required):

- Face to face

Comments on Subject Matter (Required):

- Ethics and principles appropriate for research

Comments on Faculty Participation (Required):

- Various faculty

Comments on Duration (Required):

- Annual

Comments on Frequency (Required):

- 6 hour and 8 hour courses

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 1

Research Training Plan: 2

Training Potential: 2

Institutional Environment & Commitment to Training: 1

Overall Impact: This is a strong application from a very strong applicant. The applicant hypothesizes that dsRNAs contain competing OAS1 binding sites that have different capacities to activate the protein in a context dependent manner. The aims are (1) to use complementary in vitro and human cell OAS1 activity assays to determine the features of dsRNA that lead to potent activation of OAS1 and (2) to use biochemical, biophysical and structural approaches to define the molecular mechanism(s) by which dsRNA hairpin variants differ in their effects on OAS1 activation. The applicant has taken a very careful approach in pursuit of graduate studies. Her research experience as a technician for three years provides an excellent background for the proposed project that will significantly enhance and expand the applicant's clearly demonstrated potential. The applicant is clearly committed and has strong potential to continue developing toward becoming an independent, productive scientist. The sponsor and co-sponsor bring strong mentoring skills and experience to help guide the project and applicant in her career development. Aims 1 and 2.1, 2.2 should readily yield publishable results. Aims 2.3 is higher risk, but will be significant if understanding of structure and structural changes is achieved. The proposed structure-function work will require that the applicant learn a number of new skills and possibly a significant time investment.

1. Fellowship Applicant:

Strengths

- The applicant's publication record is good, with one first author paper from a rotation and two papers (one contributing author and one co-first author) from research while working as a technician.
- The applicant has clearly demonstrated that she has the potential to become an independent, productive scientist.

- The applicant has already passed her written qualifying exam and completed most of the required coursework
- The applicant has taken a careful, mature path in pursuit of a Ph.D. Involvement in research for three years helped guide and solidify her decision. The research experience places her in a strong position to excel in her graduate studies. She is clearly committed and on an excellent path to pursue a research career.
- The applicant is described in the letters of recommendation as being goal-directed, displaying perseverance, organized, focused. One indicated that they were impressed with her mastery of her research topic and technical approaches to carry out proposed studies. She is characterized as having excellent communication and data management skills, an inherent curiosity, outstanding potential to develop into an independent scientist, well on her way to a stellar career.
- The applicant is a first generation college graduate and is described as having driven her own success to this point.
- The applicant has a good overall undergraduate GPA with mostly A-B grades, with a couple of exceptions and all A's in graduate courses. An F in organic chemistry was replaced by an A when the course was repeated.

Weaknesses

- None noted.

2. Sponsors, Collaborators, and Consultants:

Strengths

- The sponsor has a good mentoring track record, having trained eight PhD students that have ultimately gone on to academic and industry positions.
- A co-sponsor is included. Having a co-sponsor is excellent since the applicant is remaining in the same lab in which she was previously a technician. The applicant rotated in the co-sponsor's lab and developed a good working relationship with her already. The co-sponsor will provide expertise in a cell culture model for some of the activity assays. She is well funded.
- The sponsor and co-sponsor both have very strong graduate training track records.

Weaknesses

- The sponsor's current funding is a R01 for RNA modification and antibiotic resistance. This will be offset if the pending R01 that is directly related to the proposed research is funded.

3. Research Training Plan:

Strengths

- The proposed studies are based on the rationale that specific features of dsRNA contribute to the level of OAS1 activation.
- The applicant provides reasonable potential alternative outcomes and interpretations. Alternative experiments and approaches are presented. The experiments proposed in Aim 1 and also sub-aims 2.1 and 2.2 should yield publishable results.
- The amount of proposed work that is anticipated for Aim 1 and Aim 2.1 and 2.2 is feasible for a three-year timeframe.

Weaknesses

- A large amount of work may be required to obtain crystals that diffract at high resolution as proposed in Aim 2.3. There is inherent risk that this might not be achieved in the timeframe that is requested. This is recognized by the applicant. It will be worth the risk if structures of the non-activating and hyper-activating dsRNA complexes with OAS1 are obtained and these provide significant insight into the molecular details about activation. This concern is minimized somewhat by the lab's experience and previous success with structural studies.

4. Training Potential:

Strengths

- A well designed project and training plan are proposed that should allow the applicant to move toward her career goal to be an independent researcher.
- The applicant is unsure at this point about specifically which career path she will take. She wisely plans to take advantage of opportunities to learn more about career options by participating in several seminar series and career development workshop that are offered at Emory.
- The BCDB Program requires students to develop an individual development plan (IDP).
- The applicant has developed significant research experience while working as a research technician which will allow her move toward working independently and efficiently, while being guided by her mentors.
- The applicant is continuing her graduate studies in the lab where she worked as a technician, but her current research is sufficiently different and includes a broad range of new approaches and skills that should keep her on a good career trajectory. New technical skills that will be learned, include bio-layer interferometry assays, mass spectrometry and X-ray crystallography.
- The applicant will attend local, as well as national and international, meetings which will provide opportunities to present and network. She will participate in graduate program seminars and journal clubs to help strengthen communications skills. Weekly seminars and several group meetings, including a structural biology group and RNA club will provide good opportunities for the applicant to interact with and get input on her research from other labs that share common research interests.
- A statistics course for biology research will be taken.
- The applicant will obtain some teaching experience during a one-semester teaching assistantship.

Weaknesses

- The applicant will need to learn a number of new techniques, including mass spectrometry and X-ray crystallography. This may require significant time to obtain the skills that can be productively used for the proposed experiments.

5. Institutional Environment & Commitment to Training:

Strengths

- The BCDB Program requires that students develop an individual development plan (IDP) during the first year.
- The training environment and commitment to graduate training at Emory is excellent for the proposed research.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- A six-hour ethics seminar course is required at the start of the first year. Graduate students are required to attend at least four additional ethics workshops. Both are required provided by the Laney Graduate School in collaboration with the Emory Center for Ethics. The BCDB Program provides eight monthly, one-hour ethics workshops.

Comments on Subject Matter (Required):

- All of the required topics are covered.

Comments on Faculty Participation(Required):

- A large number of faculty participate in the seminars and workshops.

Comments on Duration (Required):

- Ongoing seminars and workshops beginning in the first year and continuing through the final years of graduate training is excellent.

Comments on Frequency (Required):

- The frequency of seminars and workshops throughout the graduate program provides continued exposure to relevant ethics topics as described above which meets the NIH requirement for retraining every four years.

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 1

Research Training Plan: 1

Training Potential: 1

Institutional Environment & Commitment to Training: 1

Overall Impact: The applicant was a first generation college student, who had learned how to face setbacks and overcome challenges in her sophomore years. She continued on to achieve a very good academic record, and gained experience as an undergraduate researcher. She has already co-authored a couple of publications, and first-authored a 2016 paper. These are indications of great potential to grow into an independent and productive researcher. The two sponsors also share the same opinion about the applicant. Both sponsors have strong publication and funding records, as well as graduate student mentoring experience. They also have a number of collaborators whose expertise could benefit the project. The training plan is well laid out, including mentoring undergrad students and participation in various scientific activities outside of the group (seminars, conferences, etc.)

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:

Acceptable

Comments on Format (Required):

- Adequate

Comments on Subject Matter (Required):

- Adequate

Comments on Faculty Participation (Required):

- Adequate

Comments on Duration (Required):

- Adequate

Comments on Frequency (Required):

- Adequate

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

SCIENTIFIC REVIEW OFFICER'S NOTES: Training in the responsible conduct of research was adequately described.

Footnotes for 1 F31 AI133950-01; PI Name: Schwartz, Samantha Lynne

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.