Dr. William W. Sullivan
Center for Academic
Enrichment and Outreach

UNLV | STUDENT AFFAIRS

AANAPISI STEM & McNair Scholars
Summer Research Institute 2024

Program Handbook

University of Nevada, Las Vegas
The Dr. William W. Sullivan Center
for Academic Enrichment & Outreach

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Box 452006
Las Vegas, NV 89154-2006
Campus Location: SSC-A 301

Program Overview
Administered through UNLV's Dr. William W. Sullivan Center for Academic Enrichment and Outreach (CAEO), the AANAPISI STEM, and McNair Scholars Summer Research Institute (SRI) offers eligible undergraduates in CAEO's AANAPISI STEM and McNair projects the opportunity to conduct research under the guidance of a faculty mentor. The SRI program, lasting the duration of the summer, provides students with a series of training activities and assignments designed to help students gain insight into research at UNLV. By participating in undergraduate research, students are exposed to the process of scholarly inquiry and develop a host of skills related to critical thinking, academic writing, and presenting research.

For examples of previous research papers, access the 2017 CAEO Undergraduate Research Journal.

Program Guidelines
1. There are no set hourly requirements for student-faculty research—each academic discipline lends itself to unique research hours. Hourly commitments are established through student-faculty agreements. However, if an SRI student has concerns about the hours he or she is asked to commit to research work, the student should discuss the matter with Terri Bernstein, Assistant Director for the McNair Scholars Institute & Undergraduate Research.
2. Each SRI student will receive a stipend of $2,800 to support research activities during the summer. Disbursement of the stipend occurs through three equal payments of about $933.33 rather than as a lump sum via UNLV's Financial Aid & Scholarships office. These will be issued tentatively, the first working Thursday of each month upon completion of program milestones, with the total amount of the payments being $2,800.
3. Each SRI mentor will receive incentive funds totaling $750. Note: Only persons currently employed by the Nevada System of Higher Education (NSHE) are eligible to receive incentive funds for serving as an SRI faculty mentor. While an SRI student can be mentored by a non-NSHE faculty member, that faculty member will not receive incentive funds.
4. SRI participants must use their UNLV email accounts for all SRI-related correspondence.

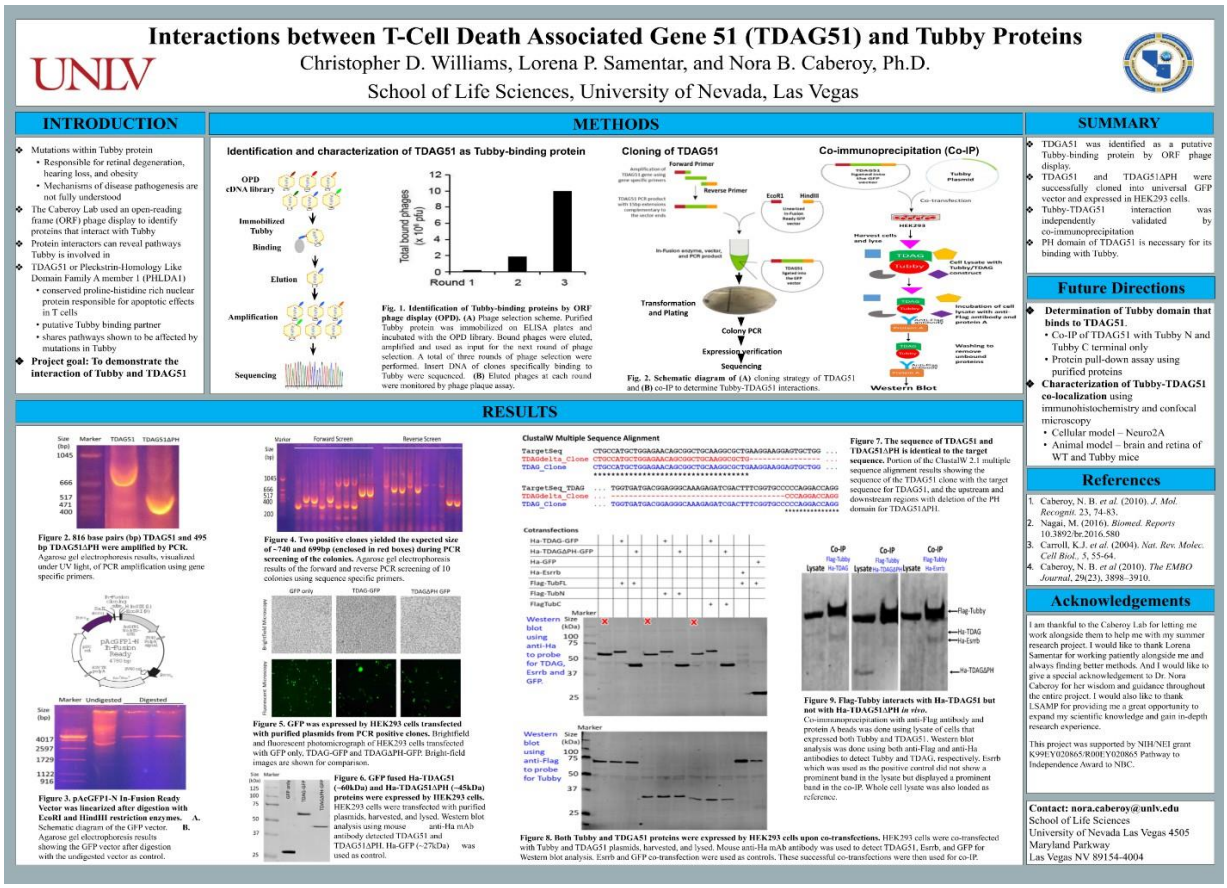
SRI Student Expectations
1. Each student must prepare a **research poster** to be presented at the CAEO undergraduate research symposium.
2. Each student must complete a full **manuscript** detailing the research conducted during the Summer Research Institute. The manuscript must be approved by the student's faculty mentor and will be published in the *CAEO Undergraduate Research Journal* (non peer-reviewed).
3. Each student must attend the **training activities** and complete the **assignments** specified in the Timeline of this *Program Handbook.*
4. Each student must participate in the monthly **peer-mentoring group meetings** (handouts are provided).
5. Each student must respond to **weekly google surveys** regarding their SRI progress.
6. Each student must participate in monthly **mandatory check-in meetings** with program staff.

SRI Faculty Mentor Expectations
1. Faculty mentors are expected to meet regularly with their mentee students to discuss their research projects.
2. Faculty mentors are expected to ensure that their mentee students receive proper guidance and supervision to successfully meet the outcomes described in the students' application/project descriptions.

Program Support
In addition to faculty mentors, the following staff members are available to provide support for students involved in research:

CAEO Undergraduate Research & McNair Scholars Institute
Terri Bernstein *Assistant Director for McNair Scholars Institute & Undergraduate Research* **Contact:terri.bernstein@unlv.edu** **Office: 702/895-4776** **Cell: 702/491-6259** **Hours:** Available by appointment **Mahya Nazarisharabian** *Graduate Assistant, McNair Scholars Institute* **Email:**mahya.nazarisharabian@unlv.edu **Cell:702/266-0573** **Hours:** Available by appointment

Samples of Research Posters



Interactions between T-Cell Death Associated Gene 51 (TDAG51) and Tubby Proteins

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UNLV

INTRODUCTION

- Mutations within Tubby protein
  - Responsible for retinal degeneration, hearing loss, and obesity
  - Mechanisms of disease pathogenesis are not fully understood
- The Caberoy Lab used an open-reading frame (ORF) phage display to identify proteins that interact with Tubby
- Protein interactors can reveal pathways Tubby is involved in
- TDAG51 or Pleckstrin-Homology Like Domain Family A member 1 (PHLDA1)
  - conserved proline-histidine rich nuclear protein responsible for apoptotic effects in T cells
  - putative Tubby binding partner
  - shares pathways shown to be affected by mutations in Tubby
- **Project goal: To demonstrate the interaction of Tubby and TDAG51**

METHODS

Identification and characterization of TDAG51 as Tubby-binding protein

Fig. 1. Identification of Tubby-binding proteins by ORF phage display (OPD). (A) Phage selection scheme. Purified Tubby protein was immobilized on ELISA plates and incubated with the OPD library. Bound phages were eluted, amplified and used as input for the next round of phage selection. A total of three rounds of phage selection were performed. Insert DNA of clones specifically binding to Tubby were sequenced. **(B)** Eluted phages at each round were monitored by phage plaque assay.

Cloning of TDAG51 | **Co-immunoprecipitation (Co-IP)**

Fig. 2. Schematic diagram of (A) cloning strategy of TDAG51 and **(B)** co-IP to determine Tubby-TDAG51 interactions.

RESULTS

Figure 2. 816 base pairs (bp) TDAG51 and 495 bp TDAG51ΔPH were amplified by PCR. Agarose gel electrophoresis results, visualized under UV light, of PCR amplification using gene specific primers.

Figure 3. pAcGFP1-N In-Fusion Ready Vector was linearized after digestion with EcoRI and HindIII restriction enzymes. A. Schematic diagram of the GFP vector. **B.** Agarose gel electrophoresis results showing the GFP vector after digestion with the undigested vector as control.

Figure 4. Two positive clones yielded the expected size of ~740 and 699bp (enclosed in red boxes) during PCR screening of the colonies. Agarose gel electrophoresis results of the forward and reverse PCR screening of 10 colonies using sequence specific primers.

Figure 5. GFP was expressed by HEK293 cells transfected with purified plasmids from PCR positive clones. Brightfield and fluorescent photomicrograph of HEK293 cells transfected with GFP only, TDAG-GFP and TDAGΔPH-GFP. Bright-field images are shown for comparison.

Figure 6. GFP fused Ha-TDAG51 (~60kDa) and Ha-TDAG51ΔPH (~45kDa) proteins were expressed by HEK293 cells. HEK293 cells were transfected with purified plasmids, harvested, and lysed. Western blot analysis using mouse anti-Ha mAb antibody detected TDAG51 and TDAG51ΔPH. Ha-GFP (~27kDa) was used as control.

ClustalW Multiple Sequence Alignment

Figure 7. The sequence of TDAG51 and TDAG51ΔPH is identical to the target sequence. Portion of the ClustalW 2.1 multiple sequence alignment results showing the sequence of the TDAG51 clone with the target sequence for TDAG51, and the upstream and downstream regions with deletion of the PH domain for TDAG51ΔPH1.

Figure 8. Both Tubby and TDAG51 proteins were expressed by HEK293 cells upon co-transfections. HEK293 cells were co-transfected with Tubby and TDAG51 plasmids, harvested, and lysed. Mouse anti-Ha mAb antibody was used to detect TDAG51, Esrrb, and GFP for Western blot analysis. Esrrb and GFP co-transfection were used as controls. These successful co-transfections were then used for co-IP.

Figure 9. Flag-Tubby interacts with Ha-TDAG51 but not with Ha-TDAG51ΔPH *in vivo*. Co-immunoprecipitation with anti-Flag antibody and protein A beads was done using lysate of cells that expressed both Tubby and TDAG51. Western blot analysis was done using both anti-Flag and anti-Ha antibodies to detect Tubby and TDAG, respectively. Esrrb which was used as the positive control did not show a prominent band in the lysate but displayed a prominent band in the co-IP. Whole cell lysate was also loaded as reference.

SUMMARY

- TDGA51 was identified as a putative Tubby-binding protein by ORF phage display.
- TDAG51 and TDAG51ΔPH were successfully cloned into universal GFP vector and expressed in HEK293 cells.
- Tubby-TDAG51 interaction was independently validated by co-immunoprecipitation
- PH domain of TDAG51 is necessary for its binding with Tubby.

Future Directions

- **Determination of Tubby domain that binds to TDAG51.**
  - Co-IP of TDAG51 with Tubby N and Tubby C terminal only
  - Protein pull-down assay using purified proteins
- **Characterization of Tubby-TDAG51 co-localization** using immunohistochemistry and confocal microscopy
  - Cellular model – Neuro2A
  - Animal model – brain and retina of WT and Tubby mice

References

1. Caberoy, N. B. *et al.* (2010). *J. Mol. Recognit.* 23, 74-83.
2. Nagai, M. (2016). *Biomed. Reports* 10.3892/br.2016.580
3. Carroll, K.J. *et al.* (2004). *Nat. Rev. Molec. Cell Biol.*, 5, 55-64.
4. Caberoy, N. B. *et al* (2010). *The EMBO Journal*, 29(23), 3898–3910.

Acknowledgements

I am thankful to the Caberoy Lab for letting me work alongside them to help me with my summer research project. I would like to thank Lorena Samentar for working patiently alongside me and always finding better methods. And I would like to give a special acknowledgement to Dr. Nora Caberoy for her wisdom and guidance throughout the entire project. I would also like to thank LSAMP for providing me a great opportunity to expand my scientific knowledge and gain in-depth research experience.

This project was supported by NIH/NEI grant K99EY020865/R00EY020865 Pathway to Independence Award to NBC.

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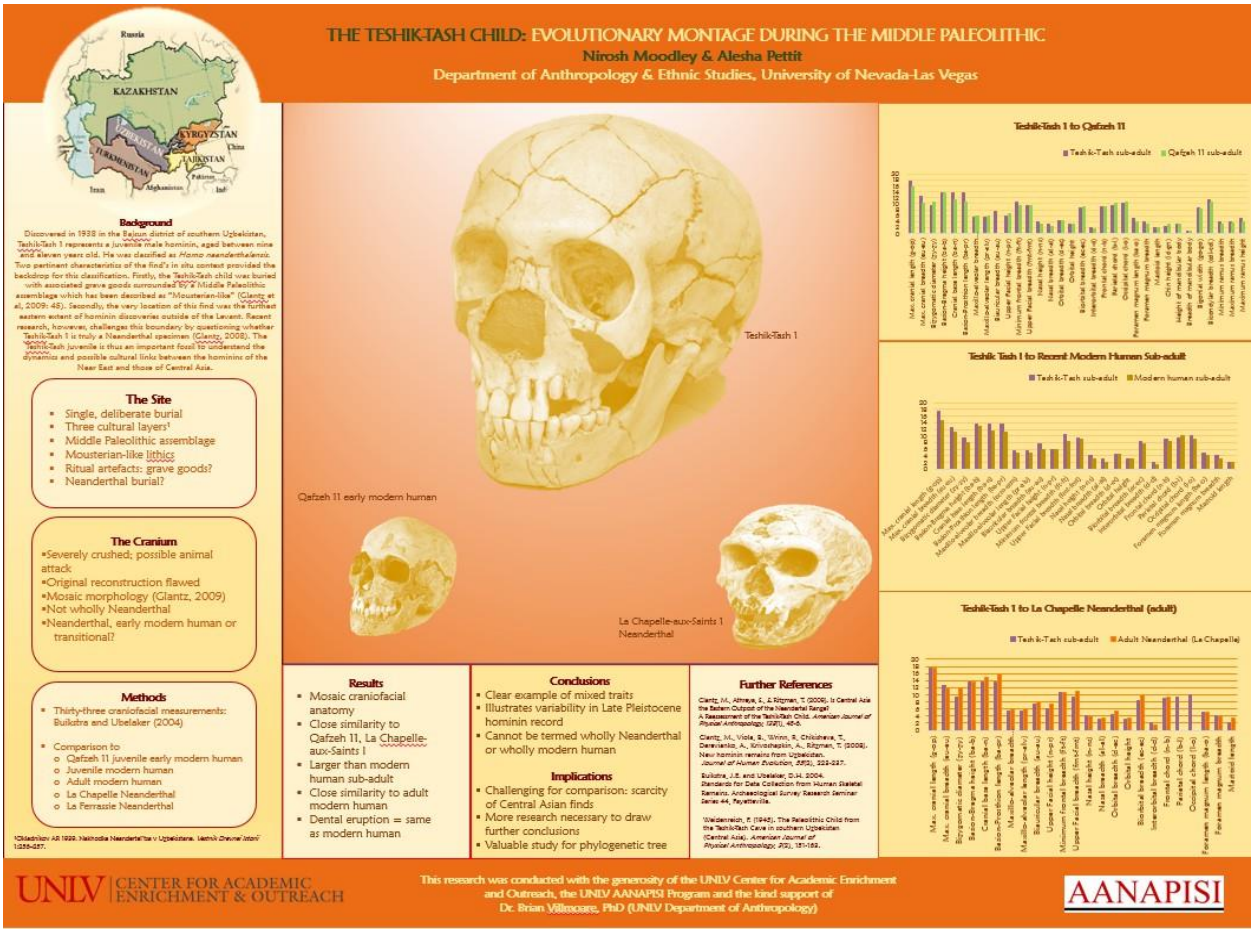

THE TESHIK-TASH CHILD: EVOLUTIONARY MONTAGE DURING THE MIDDLE PALEOLITHIC

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