Grant Proposal for the Undergraduate Research Program

Fall 2012

Shippensburg University

Biology Department

Engineering a dsRed/LC3 Expression Vector to Visualize Autophagy in Cloned Cell Lines

Faculty Advisor

Dr. Lucinda Elliott

Undergraduate Students

Biology Department Chair

Dr. Tim Maret

Relevance and Significance

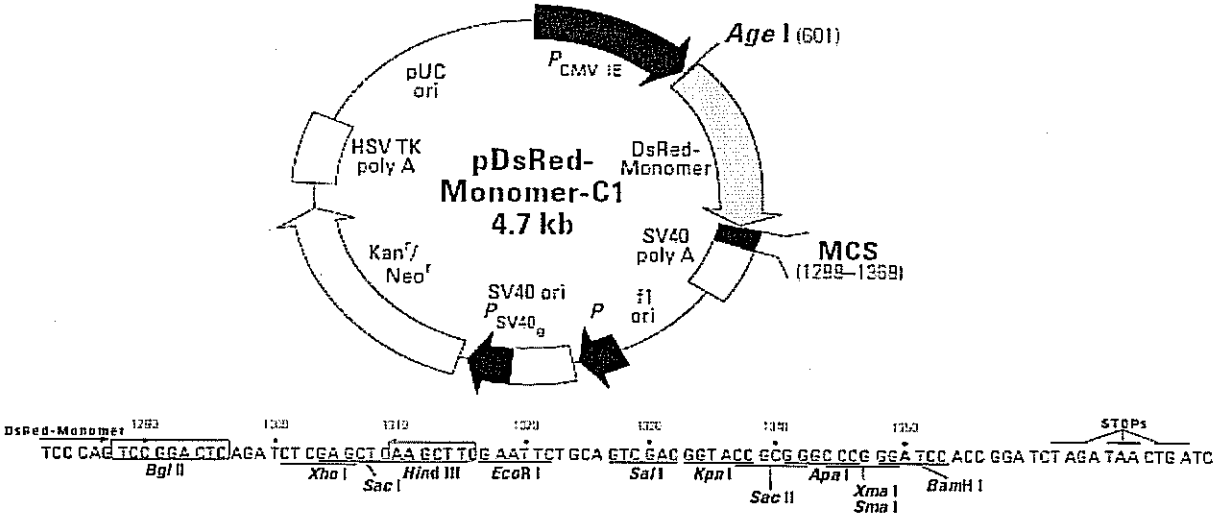
Autophagy is a fundamental cellular homeostatic process, where cells digest portions of their own cytoplasm within the lysosome in response to stress conditions. This allows the cell to adapt to environmental and/or developmental changes (1, 2). Double membrane organelles, called autopyhagosomes sequester portions of cytoplasm targeted for removal or turnover. These autophagosomes fuse with lysosomes to form autophagolysosomes, which degrade the captured cytosolic components (2). Since autophagy affects many cell types, its effects are broad; including effects on normal physiological processes like aging, or diseases like cancer and neurodegeneration. Autophagy is also linked to eliminating toxic protein aggregates and intracellular pathogens (2). Autophagy is regulated by a several evolutionarily conserved (Atg) genes that code for proteins which mediate the various steps in the process including; 1.) recruitment and isolation of cytoplasmic membrane and cargo, 2.) vesicle elongation of the double membrane to form the autophagosome around sequestered cargo and 3.) fusion of the autophagosome and associated cargo with lysosomes for degradation and recycling.

LC3 is the mammalian homolog of yeast Atg8 and to date the only identified marker for autophagsome membranes (2, 3). LC3 is a constitutively expressed microtubule-associated protein that is cleaved upon activation of the autophagy pathway, lipidated and covalently linked to membranes of developing autophagosomes. Previous experiments yielded an expression vector, which when put into cells produces a fustion protein of EGFP (enhanced green fluorescent protein)/ LC3. SNB19 cells containing this vector are currently being subcloned to obtain pure cultures that will allow visualization of autophagy as green fluorescent punctate staining (bright green spots in the cytoplasm) when EGFP/LC3 localizes to autophagosomes.

pDsRed-Monomer-C1 Vector Information

PT3796-5

Cat. No. 632466



DsRed is a monomeric red fluorescent protein that originates from Discoma sp. of reef coral (4). The DsRed tag when fused with LC3 will allow for visualization of cells participating in autophagy as red punctate staining.

Description of Project

The goal of this project is to engineer the DsRed vector (Figure 1) to express a protein containing red fluorescent protein r fused to the N terminal end of human LC3. Successful engineering of the vector and insertion of the vector into cloned cell lines will allow visualization and quantification of autophagy via the formation of fluorescent autophagosomes that appear as bright red spots (punta) in the cells. In addition, the use of a red fluorescent tag on LC3 will allow us to transfect our glioma cells containing the EGFP/Atg5 expression vector and to visualize co-localization of these two proteins (Atg5 and LC3) to autophagosomes as bright yellow spots (punta). The resulting cell line containing dsRed/LC3 as well as the double transfected (dsRed/LC3; EGFP/Atg5) cloned cell line will be used in subsequent experiments to further investigate the role of autophagy in growth of cancer cells and clearance of intracellular pathogens. The recombinant dsRed/Lc3 vector and bacterial clones containing the vector will be sent to Dr. Robert Ulrich, Chair of the Immunology Department in Integrated Toxicology at USAMRIID (United State Army Medical Research Institute for Infectious Disease) for studies on the role of xenophagy in *Yersinia* pestis (the causative agent of the Bubonic plague).

Success in this project is highly probable considering members of the team have previously engineered a similar vector, the EGFP/LC3 vector, under the guidance of the same faculty advisor.

Bacteria containing TOPO/LC3 are stored at -80 and will be used to obtain full length LC3 that is modified with BlgII and HINDIII restriction enzyme sites. who worked on the original project and has experience with these technique will lead the team. In this project, LC3 cDNA will be cut out of the TOPO-4 vector using BglII and HINDII and subcloned into the dsRed monomer C1 vector cut with the same enzymes. The dsRed/LC3 vector will then be inserted into bacteria, cloned and recombinant vector isolated for sequencing to confirm that LC3 is present, and to assure LC3 is in frame with DsRed. This vector will then be put into HEK and SNB19 glioma cells to be used for future studies which will be sent to Dr. Ulrich at USAMRIID.

Potentials for Learning

This research project will teach current techniques used in cell and molecular biology research. Students will have the opportunity to learn many of the molecular techniques used to genetically engineer an expression vector that produces a recombinant fusion protein. This vector will be used to generate genetically modified cells. These techniques are used extensively in biotechnology and biomedical research. Students will also gain extensive expertise with eukaryotic cell culture techniques. National databases and other online resources will be used and will provide experience in utilization of these tools as well. This research experience with cell and molecular techniques will better prepare the students for research based careers or acceptance into competitive Ph.D. graduate programs or professional biomedical programs that look for students who have enhanced problem solving and critical thinking skills.

Role, Involvement and Activities of Students and Faculty Mentor

. will work as team under supervision of Dr. Elliott. , previous experience with the techniques will be utilized to lead the team. Dr. Elliott will meet with team members once a week to discuss protocols, weekly research assignments and results. Dr. Elliott will instruct the students in proper laboratory techniques and instrumentation until the students gain the experience to proceed with the project without close supervision. The students will be in charge of making all solutions and performing all the steps involved in engineering the vector. If time permits the vector will be put into cell lines for future hypothesis driven experiments. Students will prepare a poster or oral presentation of their results for local and regional meetings.

References

1. Klionsky, D. Autophagy: from phenomenology to molecular understanding in less than a decade. Nature Reviews Molecular Cell Biology 2007; 1-7.

2. Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori. LC3, a mammaliam homologue of yeast Apg8p, is localized in autophagosome membranes after processing. The EMBO Journal 2000; 19; 21: 5720-5728.

3. Delgado, M., R. Elmaoued, A. Davis, G. Kyei, V. Deretic. Toll-like receptors control autophagy. The EMBO Journal 2008; 27; 7:1110-1121.

4. DsRed-Monomer fluorescent protein vectors. Westburg 2010.

Budget Justification: Item	Cost	Justification
Plastic lab supplies Sterile reservoirs (1case @ $55.00) Tissue culture flasks (25cm) (1case @ $200.00) 12 well tissue culture plates (1case @ $62.00) 15ml sterile centrifuge tubes (1case @ $100.00) **Subtotal**	 $55.00 $200.00 $62.00 $100.00 **$417.00**	Sterile plastic lab supplies will be used to subclone and maintain glioma cells transfected with the expression vectors
Cloning supplies, tissue culture & services Gel Extractor (1Pk of 100 @ $72.00) Davis sequencing Gel Red DNA stain (2@ $100.00 each) **Subtotal**	 $72.00 $50.00 $200.00 **$322.00**	Gel red DNA stain and gel extractors will be used to visualize & extract cut DNA vector and LC3 inserts for ligation and cloning. Cloned recombinant vectors will be sequenced on the LiCor DNA sequencer housed in the Biology Department and sequences confirmed by Davis Sequencing
Miscellaneous Lab Supplies Multi-channel pipettor Biohazard disposable pouches (1pk of 100) Biohazard pouch holder (1 holder) **Subtotal**	 $400.00 $75.00 $18.00 **$493.00**	The multichannel pipettor will be used for subcloning transfected cell lines. Biohazard pouches will be used for disposal of pipetted tips.
Poster printing	**$40.00**	Cost of printing on Department of Biology poster printer
Travel to PAS annual meeting (April 6, 2013) Student registration & food (3 @ $82/student) Travel (Bradford PA- 190miles @ 0.555/mi) Lodging **Subtotal**	 $246.00 $104.45 $120 **$471.45**	Requesting funds for all three students. Students will share rooms (4 to a room) with other students; Estimate at least 2-3 rooms at $120 each
Total	**$1743.45**	
Faculty Registration with two meals Hotel will share with another faculty member	$110 75 **$185**	

2012-13 UNDERGRADUATE RESEARCH GRANT PROGRAM

FOR IPSSP USE

Shippensburg University of Pennsylvania: This worksheet is designed to assist you in preparing your proposal budget. Work with your faculty mentor to identify reasonable costs. Enter your information in the "pink" shaded areas. If you prefer, you may create your own worksheet. If you do so, you are expected to use the same budget categories and to use the same layout as provided on this template. Call IPSSP for questions and/or technical assistance: 717-477-1251.

Project Title:	**Engineering a dsRed/LC3 Expression Vector to Visualize Autophagy in Cloned Cell Lines**				
Lead Student Name:					
Lead Student ID #:					
	UGR Grant - Proposed Budget				
			$/unit	#	$
Supplies	Item	*Example*	25	3	75
	Item 1	Sterile reservoirs	55	1case	55
	Item 2	Tissue culture flasks (25cm)	200	1case	200
Use these lines for consumable supplies and materials you will need for your project. **Do not** request funding for electronic equipment and/or digital devices already available through the University.	Item 3	12 well tissue culture plates	62	1case	62
	Item 4	15ml sterile centrifuge tubes	100	1case	100
	Item 5	Gel Extractor	72	1pkg	72
	Item 6	Gel Red DNA stain	100	2 vials	200
	Item 7	Multi-channel pipettor	400	1 each	400
	Item 8	Biohazard disposable pouches	75	1pkg	75
	Item 9	Biohazard pouch holder	18	1 each	18
				TOTAL SUPPLIES	1182
					$
Printing/Posters	Item 1	Poster printing Dept of Biology printer			40
	Item 2	*description*			
	Item 3	*description*			
				TOTAL PRINTING	40
					$
Student Travel	**Registration includes two meals (3 students @ $82)**				246.00
	Air fare				
	Bradford, PA 190mi x 0.555				104.45
	Tolls				
	Parking				
	Hotel				120.00
	Meals	(maximum = $25/day; itemized receipts required)			
	Other				
				TOTAL STUDENT TRAVEL	471.45
					$
Other	*Confirmation of sequences by Davis Sequencing*				50
Use for expenses that do not fit categories above.	*description*				
				TOTAL OTHER	50
					$
Faculty Travel	Registration with two meals				110.00
	Air fare				
NOTE: The maximum faculty award is $400.	Automobile miles @ 0.555/mile				
	Tolls				
	Parking				
	Hotel				75
	Meals	(maximum = $25/day; itemized receipts required)			
	Other				

TOTAL FACULTY TRAVEL	185
TOTAL STUDENT REQUEST	1743.45
TOTAL FACULTY REQUEST ($400 max)	185.00

Consumable supplies are those which will be used-up or spent during your project. (Durable equipment items – including electronics and digital devices – will not be funded unless they are unavailable through the University. Such items must be retained by the University upon project completion.)