UW – Green Bay Research Council

Grants for Integrating Research and Teaching
Cover Page

Name: Uwe Pott

Phone: 465-2282

Email: pottu@uwgb.edu

Address: LS-453

Department: Human Biology

Unit: NAS

Project Title: Identification of the rKr2 Nuclear Localization Signal in the Molecular Biology Lab Course

Amount Requested: $ 1342.51

Anticipated Dates: Fall semester of 2007

Proposals should include:

☐ Proposal Narrative
- State the nature of the research activity
- State the number of students expected to participate
- State the significance of student participation
- Five page limit

☐ Budget of Expenses
- Include a summary of how funds will be spent
- One page limit

☐ Curriculum Vitae
- Limited to relevant scholarly work
- Two page limit

Need

Student participation in an original research project should be an integral part of any life science curriculum. This type of experience gives students a taste of how "real" science is done and it gives them an edge when applying for jobs in industry or for admission to graduate and professional academic programs. Consequently, Human Biology and Biology at UWGB offer individual students the opportunity to join faculty members in their research endeavors (HumBiol 495 – Research in Human Biology; Biology 495 – Research in Biology). It is obvious, though that of the more than 400 majors in Human Biology alone only a relatively small fraction will be able to make use of this offer. I therefore propose testing the feasibility to perform original experimental research in one of my laboratory courses, Biology 408 – Molecular Biology Lab.

Project

Every cell of a multicellular organism contains the same genes, yet only a subset of these genes is expressed in each cell type (liver cell, nerve cell, etc.). Gene expression is controlled by a large set of transcription factors, proteins that are imported from the cytoplasm into the nucleus of a cell, where they bind to the DNA and activate or repress the transcription of genes. For entering the cell's nucleus a protein needs a "key", a so-called nuclear localization signal (NLS). Whereas some of these signals have been known for many years now, the identity of the NLS of some nuclear proteins is still unknown. For example, two zinc finger proteins, named rKr1 and rKr2, which I have identified during my Ph.D. and postdoctoral work do not contain any of the known nuclear localization signals. These proteins belong to a large class of transcription factors and they are clearly imported into the nucleus. Therefore, a logical research project is to identify the nuclear localization signals of the rKr1 and rKr2 proteins and determine the minimal amino acid sequence capable of directing nuclear localization in these proteins. I plan to have students of the Molecular Biology lab perform this analysis for the rKr2 protein in the fall of this year.

Activities and Outcome

The successful outcome of this research project is the identification of the minimal part of the rKr2 protein that is necessary and sufficient to cause nuclear localization of this protein. However, along the way there are several stages that have to be completed successfully. For instance, recombinant DNA molecules have to be generated in order to express parts of the rKr2 protein in a mammalian cell line. Some of these constructs may not define the *minimal* NLS, but they will certainly either contain the NLS or not. Therefore, even if the final goal of the project may not be achieved in one lab course, students will have the satisfaction of completing landmark steps toward this goal.

The goal of an upper-level lab course has to be giving students an opportunity to perform state-of-the-art experimental procedures within the field of study. This, of course, can be done with canned experiments, the outcome of which is known and described, along with the experimental background and approach, in a cookbook-style lab manual. Another possibility is the subject of this proposal, namely involving students in an original research project that will collect new, possibly publishable data. Beyond training them in experimental techniques, this second approach will give students the pleasure of having made a contribution to answering an unsolved scientific question. However, this must not come at the cost of sacrificing breadth of students' training. The proposed study will give my students ample opportunity to apply a wide range of relevant techniques. In order to achieve the project goal as outlined above, students will: 1) study background literature on nuclear localization and zinc finger proteins, 2) involve bioinformatics software to plan the construction of appropriate recombinant DNA molecules, 3) perform sub-cloning of DNA fragments in the test tube, 4) verify the constructs with restriction endonulease analysis, 5) perform large-scale DNA isolation from bacterial cells, 6) practice the handling of a mammalian cell line and transfect the cells with the recombinant DNA, and 7) view the

transfected cells under the fluorescent microscope and document the expression of proteins from the recombinant DNA.

Obviously, the proposed project has a self-serving component as well. Advancing one's research with a handful of students every year or so, is a painfully slow process. Focusing the man- and womanpower of at least twelve students in a single lab course on original research may be a way to move ahead with my scholarship more quickly. It seems to me that a project like this is a win-win situation for both, my students and me.

Work Plan

At the beginning of the course I will ask my students to study the relevant literature on nuclear localization signals and zinc finger proteins, including the rKr2 protein. They will have to extract pertinent DNA sequence information from databases in order to plan the generation of recombinant DNA molecules that direct the production of rKr2 fusion proteins in mammalian cells. As a fusion partner for the rKr2 fragments I have chosen green fluorescent protein (GFP) which is available in several standard cloning vectors. Due to its bright fluorescence under UV light, GFP can be easily detected under a fluorescence microscope, when it is expressed in cells. Therefore, pieces of the *rKr2* gene will be cloned in-frame with GFP, and the expression of the fusion proteins in COS7 cells will be observed making use of the fluorescent GFP tag of these proteins. The fragments of rKr2 will or will not contain an NLS so that the fluorescence will be either found in the cytoplasm or the nucleus of the cells. Covering the whole rKr2 protein piece by piece will reveal the location of the NLS in the protein. During this phase of the project students will use the pDRAW32 program to identify appropriate fusion constructs; this planning stage is expected to last for about three weeks.

The next step will be the actual generation of the fusion constructs. Restriction fragments of the *rKr2* gene will be cloned into a GFP containing cloning vector such that the reading frame of the zinc finger protein will continue into the reading frame of GFP. The production

and purification of the restriction fragments, their ligation with the cloning vector, and the propagation of the recombinant DNA molecules in *Escherichia coli* cells should take about four weeks. The restriction analysis of the fusion constructs and their large-scale purification from the bacterial cells will take another three weeks.

In the final stage of the project, which will comprise five weeks of the lab course, the students will practice growing a mammalian cell line (COS7) in culture. Due to their relatively low growth rates, mammalian cells are very vulnerable to microbial contamination; therefore the students will have to learn proper sterile techniques. This phase will also include the transfection of COS7 cells with the recombinant rKr2-GFP constructs using the polyethylenimine procedure and the observation of GFP fluorescence under the microscope. For documentation of the results, images of GFP expressing cells will be taken with the digital camera connected to our fluorescence microscope.

Evaluation and Assessment

The evaluation of success in a research project such as this one, with clearly defined objectives, is straight forward. The analysis at the end of each stage of this project, performed by the students themselves, will show if the corresponding goal was reached. For the assessment of student learning, on the other hand, I will evaluate a lab notebook kept by each student throughout the semester and a final poster presentation of the obtained results at the end of the lab course. Both products will enable me to assess student engagement with the project and depth of understanding of the subject matter. Of course, I will also observe participation in the planning stage of the project and hands-on experimental skills of individual students. Finally, as to understand the satisfaction of the students with this new lab format, a detailed questionnaire will be developed and handed out to them together with the usual CCQ form.

Dissemination

At the least, two to four students, depending on class enrollment and the resulting group sizes, will present their findings at the Academic Excellence Symposium in the spring of 2008. I envision a situation in which I will nominate students for the symposium based on the best poster presentation in the lab, adding an extra incentive to perform well in the course. Of course, publication of the data in a peer-reviewed research journal is the ultimate goal of this work. In addition, I will forward a report to the research council by the end of June 2008, describing the success of the research project and the viability of this new lab format from my and my students' perspective.

Summary

This proposal describes a novel format for my upper-level Molecular Biology lab course, in which students are involved in original experimental research rather than performing pre-fabricated experiments of known outcome. Based on previous enrollment in this course, I anticipate that about 12 students will participate in research on the nuclear localization of the rKr2 zinc finger protein during the upcoming fall semester. However, if this lab format proofs to be successful, a significant larger number of students will profit from it in the future. For example, I could have students of the Genetics lab investigate the nuclear localization signal of the rKr1 zinc finger protein in the spring of 2008. The typical enrollment of this lab course is about 20 students. Moreover, in semesters to come I would use the same basic research format for my upper-level labs, breaking up my scholarly interests into questions that can be answered in a semester-long lab course. Finally, if successful this model may motivate colleagues in the sciences to adopt it for their own upper-level labs.

Budget

Item	**Manufacturer**	**Price**
8 restriction endonucleases	Promega	$ 400.00
Fetal Bovine Serum, 200 ml	HyClone	$ 188.00
DMEM cell culture medium, 2 l	BioWhitaker	$ 69.60
Cell culture flasks, 25 cm^2, Case of 200	Corning	$ 309.91
Cell culture flasks, 75 cm^2, Case of 100	Corning	$ 273.80
6 well cell culture plates, Case of 50	Becton Dickinson	$ 101.20
	Total	**$ 1342.51**

Cloning vectors containing the green fluorescent protein are available from my previous research. Some restriction enzymes and disposables such as reaction tubes, pipette tips etc. are available as well. However, additional restriction enzymes will most likely have to be purchased, depending on cloning strategies developed by the students. Based on previous experience, my best “guestimate” is that we will need about 8 more enzymes. Taking into account that different restriction enzymes vary tremendously in price, this budget line should be considered a rough estimate. Most of the grant money will be spent on cell culture materials, of which we carry only a very small stock. Expenses other than these materials and supplies for the proposed project are not expected.

UWE POTT

CURRICULUM VITAE

PERSONAL

Address:	Human Biology
	University of Wisconsin-Green Bay
	LS-455
	2420 Nicolet Drive
	Green Bay, WI 54311-7001
	Phone: 920-465-2282
	Fax: 920-465-2769
	E-mail: pottu@uwgb.edu

EDUCATION

1976 - 1987:	Biology study at the Heinrich Heine University, Düsseldorf, Germany Degree: Dipl. Biol. (Microbiology)
1988 - 1994:	Doctoral study at the Brain Research Institute, University of Zürich, Switzerland Degree: Ph.D. (Molecular Biology)

WORK EXPERIENCE

1987 - 1988:	Research associate, Institute of Physiological Chemistry I, University of Düsseldorf
1995 - 1998:	Visiting fellow at the Laboratory of Developmental Neurogenetics, NINDS, NIH in the group of Dr. Lynn Hudson
1998 - 1999:	Visiting associate at the Laboratory of Developmental Neurogenetics, NINDS, NIH in the group of Dr. Lynn Hudson
1999 - 2005:	Assistant Professor of Human Biology at the University of Wisconsin-Green Bay
2006 - present:	Associate Professor of Human Biology at the University of Wisconsin-Green Bay

LIST OF PUBLICATIONS

Research Articles

Wagner, G., **U. Pott**, M. Bruckschen, and H. Sies. 1988. Effects of 5-azacytidine and methyl-group deficiency on NAD(P)H: quinone oxidoreductase and glutathione S-transferase in liver. Biochem. J. 251:825-829.

Müller, F., W. Laufer, **U. Pott**, and M. Ciriacy. 1991. Characterization of products of TY1-mediated reverse transcription in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 226:145-153.

Pott, U., N. Schaeren-Wiemers, C. Schaefer, and M. E. Schwab. 1991. A molecular biological approach to the regulation of oligodendrocyte differentiation. Schweiz. Arch. Neurol. Psychiatr. 142:123-126.

Fuss, B., **U. Pott**, P. Fischer, M. E. Schwab, and M. Schachner. 1991. Identification of a cDNA clone specific for the oligodendrocyte-derived repulsive extracellular matrix molecule J1-160/180. J. Neurosci. Res. 29:299-307.

Colello, R. J., **U. Pott**, and M. E. Schwab. 1994. The role of oligodendrocytes and myelin on axon maturation in the developing rat retinofugal pathway. J. Neurosci. 14:2594-2605.

Pott, U. and B. Fuss. 1995. Two-color double *in situ* hybridization using enzymatically hydrolysed nonradioactive riboprobes. Anal. Biochem. 225:149-152.

Moser, M., R.J. Colello, **U. Pott**, and B. Oesch. 1995. Developmental expression of the prion protein gene in glial cells. Neuron 14:509-517.

Pott, U., H.-J. Thiesen, R.J. Colello, and M.E. Schwab. 1995. A new Cys_2/His_2 zinc finger gene, *rKr2*, is expressed in differentiated rat oligodendrocytes and encodes a protein with a functional repressor domain. J. Neurochem. 65:1955-1966.

Colello, R.J., L.R. Devey, E. Imperato, and **U. Pott**. 1995. The chronology of oligodendrocyte differentiation in the rat optic nerve: evidence for a signalling step initiating myelination in the CNS. J. Neurosci. 15:7665-7672.

Hasan, S.J., **U. Pott**, and M.E. Schwab. 1995. Transcription of a new zinc finger gene, *rKr1*, is localized to subtypes of neurons in the adult CNS. J. Neurocytol. 24: 984-998.

Holz, A., N. Schaeren-Wiemers, C. Schaefer, **U. Pott**, R.J. Colello, and M.E. Schwab. 1996. Molecular and developmental characterization of novel cDNAs of the Myelin-associated/Oligodendrocyte Basic Protein. J. Neurosci. 16:467-477.

Pott, U., R.J. Colello, and M.E. Schwab. 1996. A new Cys_2/His_2 zinc finger gene, *rKr1*, expressed in oligodendrocytes and neurons. Mol. Brain Res. 38:109-121.

Lovas, G., W. Li, **U. Pott**, T. Verga, and L.D. Hudson. 2001. Expression of the *Krüppel*-type Zinc Finger Protein rKr2 in the Developing Nervous System. Glia 34, 110-20.

Reviews

Colello, R.J. and **U. Pott**. 1997. Signals that initiate myelination in the developing mammalian nervous system. Mol. Neurobiol. 15:83-100.