3-29-2006

Biochemical and Molecular Autonomy of Symbiotic Chloroplasts

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Final Report for Period: 02/2001 - 01/2006
Submitted on: 03/29/2006
Principal Investigator: Rumpho, Mary E.
Award ID: 0095129
Organization: University of Maine
Submitted By:

Title:
Biochemical and Molecular Autonomy of Symbiotic Chloroplasts

Project Participants

Senior Personnel

Name: Rumpho, Mary
Worked for more than 160 Hours: Yes
Contribution to Project:
Mary Rumpho is the PI on this grant. Her salary was provided by the Univ. of Maine for the entire year of the grant.

Name: Manhart, James
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Manhart was a co-PI on the grant and received support through a sub-contract. He is responsible for Objective 3 which includes sequencing and mapping the chloroplast genome of Vaucheria litorea. Dr. Manhart's salary is provided by Texas A&M University and 2 months summer salary from this grant.

Post-doc

Name: Dastoor, Farahad
Worked for more than 160 Hours: Yes
Contribution to Project:
Dr. Dastoor began working on this project October 1, 2001. He is focusing on Obj. 1 related to protease activity in the alga vs. the mollusc. He is being funded from this grant.

Name: Li, Jungho
Worked for more than 160 Hours: Yes
Contribution to Project:
Jungho Li is working on completing the sequencing of the chloroplast genome of Vaucheria litorea. He was partially supported by this grant.

Graduate Student

Name: Rollins, Jarod
Worked for more than 160 Hours: Yes
Contribution to Project:
Jarod was a graduate student in the Dept. of Biochemistry, Microbiology and Molecular Biology until June 2002 and worked on Obj. 1 of this project. Jarod was funded by departmental monies.

Name: Pochareddy, Sirisha
Worked for more than 160 Hours: Yes
Contribution to Project:
Sirisha has been working as a graduate assistant focusing on identifying algal genes in the sea slug nucleus and also on protein stability. She is supported on a departmental teaching assistantship.

Name: Nayak, Akshata
Worked for more than 160 Hours: No
Contribution to Project:

Akshata rotated through my lab fall semester as part of the required new grad student rotation. She began investigating whether the sea slugs and/or alga produces any anti-microbial compounds. She was supported by a departmental teaching assistantship.

Name: Worful, Jared

Worked for more than 160 Hours: Yes

Contribution to Project:

Jared finished his undergrad degree and began his MS degree Sept. 2005. He is supported by a University research assistantship.

Undergraduate Student

Name: Needham, Alex

Worked for more than 160 Hours: Yes

Contribution to Project:

Alex is Junior in the Dept. of Biochem., Microbiol. and Molecular Biology. He is working on characterizing protein turnover and proteases in the sea slugs and algae. He is supported by NSF funds and state funds.

Name: Bateman, Janet

Worked for more than 160 Hours: Yes

Contribution to Project:

Janet graduated from UMaine with a BS in Biochemistry in June 2002. Until that time she had been working on algae culturing and protein synthesis in the algae and sea slugs. She was supported by state funds.

Name: Benoit, Michelle

Worked for more than 160 Hours: Yes

Contribution to Project:

Michelle is a first year biology student and began working in my lab in the summer and continuing in the school year. She has worked on chloroplast isolation and studying their stability in isolation. She will be applying for an ASPB SURF fellowship to continue her research.

Name: Worful, Jared

Worked for more than 160 Hours: Yes

Contribution to Project:

Jared is a 2nd year student and is working on purifying a virus from the sea slugs to identify the type of virus and to determine if it could be involved in horizontal gene transfer. This is Jared's second year working in my lab. He is supported by federal work-study funds and my state money.

Name: Morrison, Jayson

Worked for more than 160 Hours: Yes

Contribution to Project:

Jayson is completing his senior capstone research in my lab. He is studying the effect of algal feeding on adult sea slugs to determine if chloroplasts are continually taken up and their longevity.

Name: Cheng, Chen

Worked for more than 160 Hours: Yes

Contribution to Project:

Chen is completing her senior capstone research project on the symbiosis project. She has been attempting to identify genes in the sea slug which presumably were transferred from the algal nuclear genome over the course of evolution.

Name: Refsland, Dane

Worked for more than 160 Hours: Yes

Contribution to Project:

Dane was suppored by an NSF REU fellowship during the summer of 2003. He specifically studied nitrate reduction in the sea slugs and algae and attempted to PCR the gene for nitrate reductase.

Name: Soule, Kara

Worked for more than 160 Hours: Yes

Contribution to Project:

Kara began working on this project as an undergraduate research student fall of 2005. She was paid from this grant as well as other private grants.

Technician, Programmer

Other Participant

Research Experience for Undergraduates

Organizational Partners

Texas A&M University Main Campus

Drs. Rumpho (UMaine) and Manhart (TAMU)are collaborating on this research project and Dr. Manhart has a sub-contract through this grant. Dr. Jungho Li was a Post-doctoral Associate in Dr. Manhart's laboratory at TAMU, but has now moved to a permanent position in South Korea. He returns to TAMU periodically and continues to collaborate on the project and is working on finishing up manuscripts from his work.

Other Collaborators or Contacts

I have an ongoing collaboration with Dr. Kathleen Archer at Trinity College in Hartford, CT, a predominantly teaching college to investigate behavioral aspects of the sea slug/algal symbiosis as well as to characterize the digestive system of the sea slug and chloroplast uptake processes.

Early in the project, I collaborated with Dr. Michael Salvucchi at the Univ. of Arizona to investigate photosynthetic aspects of the symbiosis.

Under the direction of Dr. Douglas Zook at Boston University, I was a participant on a planning grant submitted to NSF to form an international research group focused on symbiosis. This grant was not funded.

Early in 2005,I began collaborating with Dr. Mary Tyler, Biology Dept., University of Maine. We are working on developing educational materials related to sea slug development.

In 2005 I began collaborating with Mr. Soren Hansen, a PhD student in the marine biology program at the University of Maine. Soren is developing a commerical aquaculture system for clownfish and is assisting us with the culturing of sea slugs in the Aquaculture Research Center on campus.

Activities and Findings

Research and Education Activities:

Elysia chlorotica is a marine sea slug which harbors, intracellularly, intact chloroplasts from the chromophytic alga Vaucheria litorea. These plastids remain functional for up to 10 months despite the absence of an algal nucleus. Considering the dependency of plastid function on nuclear genes, the level of chloroplast activity observed in the animal cell is quite remarkable. Possible factors contributing to this long-lasting functional association which we are studying include: autonomous chloroplasts, unusual chloroplast/protein stability, re-directing of animal proteins to the chloroplast, and lateral gene transfer.

Specific Objectives:
1. Characterize the structural and functional long-term stability of isolated V. litorea plastids, the stability of plastid proteins, and the activity of chloroplast proteases in sea slugs vs. algae.
2. Determine if the sea slug nuclear genome codes for and targets any proteins to the chloroplast and the general mechanism of protein import in chromophytic plastids.
3. Elucidate the genetic autonomy of the symbiotic chloroplasts by mapping and sequencing the chloroplast genome of Vaucheria litorea.
4. Additional activity developed...Develop a system to culture the sea slugs through their entire life-cycle in the lab, including the establishment of the chloroplast symbiosis.

Experimental:
Obj. 1a: To examine chloroplast stability, algal chloroplasts were isolated using a two step Percoll gradient (30%/75%) and mollusc-sequestered chloroplasts were isolated in a modified isolation buffer and a 50% linear Percoll gradient. For the 72 h time-courses, chloroplasts were pelleted and resuspended in fresh isolation buffer (minus BSA) after each time point to avoid bacterial contamination and stored at 4C in the dark throughout. Intactness was evaluated first by phase-contrast microscopy. Twenty images (40X obj.) were captured per slide (10 mg chl) and analyzed using Image J (NIH) to allow for intensities, measured by pixels, of the chloroplast membrane 'halos' to be determined. Functional stability was assayed by FeCN-dependent O2 evolution activity and used to estimate both intactness and functional ability along with light-dependent fixation of NaH14CO3 and in vitro translation of chloroplast proteins. In vitro translation reactions were carried out in a reaction mix of: 50 mCi [35S]Met, 0.5 mM amino acids (- Met), and chloroplasts (80 mg) under 800 mmol photons s-1 m-2 or in the dark (control), at room temperature. Thylakoid and stromal fractions were isolated by differential centrifugation following lysis. Polypeptides were separated by SDS-PAGE (9 to 18% w/v linear gradient). Samples were loaded on an equal radioactivity (100,000 dpm) or equal volume basis. A manuscript was published on these studies.

Obj. 1b. Protein stability was examined by labeling algal filaments and sea slugs with 35S-Met for 4 hrs and following the stability of labeled proteins over a 2 month chase period (with cold Met) by SDS-PAGE fluorography.

Obj. 1c. Chloroplast protease activity is being compared in algae and sea slugs using western blotting and antibodies to a number of heterologous chloroplast proteases (FtsH, ClpC, DegP) from diatoms.
We are currently evaluating their role in regulating chloroplast protein stability.

Obj. 2c: Phosphoribulokinase (PRK) activity was measured in homogenized algal and animal extracts spectrophotometrically at 25 C in an assay that couples Ru5P-dependent ADP formation by PRK to the oxidation of NADH via pyruvate kinase (PK) and LDH (PEP to lactate). Chloroplast isolation and Western blotting were performed as previously described.

For gene cloning and Southern blotting, gene fragments of V. litorea PRK, PetC and LHCP were isolated by RT-PCR. PRK and LHCP 3' and 5' sequences were obtained by RACE cloning. DNA was isolated from E. chlorotica and V. litorea. All fragments were cloned into pGemT-Easy and transformed into JM109 cells. The probes for chloroplast encoded Rubisco and V. litorea genomic DNA specific internal transcribed spacer region (ITS) have been described previously and Southern blotting was by standard protocols.

Obj. 3: Purified V. litorea ctDNA was cut with PstI and 'shotgun' cloned using Bluscript KS+. Cloned fragments have been sequenced in their entirety using primers to the vector followed by a combination of primer walking and sequencing subclones in which transposons containing universal priming sites have been inserted at random (Genome Priming System,New England Biolabs). Sequencing was done at the Univ. of Maine DNA sequencing facility and the TAMU Biological Sciences sequencing facility. Sequencher software from ABI and Vector NTI Suites software is used to analyze and organize the DNA sequences and the identity of genes determined by comparison with known sequences using Blast.

New objective 4. Chloroplast-free, non-pigmented, sea slug eggs were collected from aquaria in the lab and maintained in petri plates under sterile conditions. Developmental stages were observed and recorded. Upon hatching of the veligers from the egg cases, unicellular algae were provided as a food source. Upon further development to the juvenile sea slug stage, filamentous Vaucheria litorea was provided to promote metamorphosis to the adult stage and initiate the chloroplast symbiosis. We had never succeeded in the past in accomplishing this task.

Presentations made:
The PIs and their students routinely present their research findings at the annual meeting of the American Society for Plant Biologists (ASPB). In 2002, we presented as part of an invited symposium at the ASPB meeting in Denver, Co. In addition, seminars were presented at the University of New Hampshire and Boston University in 2002, College of the Atlantic, Bar Harbor, ME, in 2004, the Univ. of New Brunswick in 2005, and the University of Maine in 2004 and 2006. In August of 2003, our results were presented in an oral talk and through a student poster at the International Symbiosis Society (ISS)Congress held in Halifax, Nova Scotia. Dr. Rumpho is helping organize and will be a keynote speaker at the ISS Congress in Vienna, Aug. 2006. Finally, Dr. Rumpho was an invited keynote speaker at the FEBS Advanced Lecture course on, 'The Origin and Evolution of Mitochondria and Chloroplasts,' in Germany, March 2005. An undergraduate also presented a poster at this conference. Dr. Rumpho has been invited to present again at the FEBS conference in spring 2007.

Products:
Several publications have resulted from this work or are in progress; several gene sequences have been deposited in GenBank; and a teaching exhibit has been established at Mary Hurd Elementary School, N Berwick, ME, in collaboration with teacher, Ms. Cindy Langdon. In addition, the PI has secured an educational grant from the American Society of Plant Biologists to develop educational materials related to this project and a grant from the Maine Technology Institute to explore the possibility of commercializing the sea slugs for education, research and reef

aquaria hobbyists.

Findings:

Objective 1a. To determine if V. litorea chloroplasts display unusual stability or 'robustness' which may contribute to their successful, long-term association within the mollusc, we characterized isolated plastids over a 72 h time-course. Structural integrity was evaluated by phase-contrast microscopy with Image J analysis and also by transmission electron microscopy. Functional stability was evaluated by measuring FeCN-dependent O2 evolution, light-dependent fixation of NaH14CO3, and in vitro translation of chloroplast proteins. Upon isolation, both algal and sea slug plastids exhibited at least 80% intactness and remained at greater than 50% intact after 72 h as determined by phase contrast microscopy. FeCN-dependent oxygen evolution revealed an intactness of about 65% at 48 h and 25% at 72 h which also reflected 14CO2 incorporation rates. Translational activity of isolated algal chloroplasts persisted for the entire 72 h and the number of thylakoid and stromal proteins synthesized did not change. Both Rubisco LS and D1 protein were synthesized throughout, although the intensity declined at 72 h. TEM revealed that the plastids are surrounded by chloroplast endoplasmic reticulum (ctER) in the alga, but this is lost upon isolation or uptake by the sea slug. From these results we concluded that the algal chloroplasts are more 'robust' than typical spinach plastids and this may aid at least in the uptake and incorporation of the plastids into the animal cytosol and possibly also in long-term acclimation to the foreign environment. We have carried out additional longer-term studies on isolated V. litorea plastids and have found that they remain over 50% intact, as observed by phase-contrast microscopy, for as long as 20 days. These results were published in a manuscript in Symbiosis, 2005.

Objective 1b. Several proteins were labeled in algal filaments and whole sea slugs incubated with 35S-met for 4 hours. The polypeptide patterns remained very similar over a 2 month chase in the sea slugs, whereas, considerable loss of radio-labeled polypeptides was noted in the algal extracts. The general decline in specific activity in the alga could be explained by the growth which occurred over the 2 month period; but the qualitative changes cannot be explained by this. The labeling experiments need to be repeated with modifications to optimize the isolation procedures for chloroplasts after pulse/chase periods to analyze only the chloroplast proteins. This should also help in further concentrating radiolabeled PRK protein for identification and analysis.

Objective 1c. From our chloroplast sequencing data we found that V. litorea plastids, like other chromophyte plastids, encode at least two proteases nuclear encoded in higher plants, FtsH (ycf25) and ClpC. These two proteases have been implicated in the degradation of mal-folded, unassembled, and/or inactive proteins. However, we have not found the regulatory subunit, ClpP, in the plastid genome, whereas, it is found in the plastid genome of plants. We have confirmed by western-blot analysis the presence within the sea slug of ClpP/ClpC and FtsH proteins.

Objective 2c. The only enzyme of the Calvin Cycle predicted to be nuclear encoded in a chromophyte and with no obvious homologue in animals is phosphoribulokinase (PRK). Immunoblot analysis indicated that PRK levels in isolated chloroplasts from 9 month old sea slugs were very similar to levels in rapidly growing algal filaments and only slightly less than in spinach chloroplasts. Levels in 9 month old animals were also very similar to that found in total animal extracts at 3 months. PRK activity was detectable in extracts of E. chlorotica assayed at 5 months. The specific activity was about 10-fold less than algal extracts; possibly a reflection of a decrease in the ratio of PRK to total proteins in the animals. Using tobacco antiserum to phosphoribulokinase (PRK), we have preliminary evidence based on western blot analysis of radiolabeled proteins for the de novo synthesis of PRK in the sea slug kleptoplasts after 4 months association. In addition to PRK, we identified three other algal nuclear encoded proteins by activity or immuno-staining including, a light harvesting complex protein (Lhcp), a Reiske Fe/S protein (petC) and the water splitting accessory protein, psbO, which are present in 9 month old symbiont chloroplasts.

Having shown that 9 month old sea slugs contain PRK, Lhcp and psbO proteins, but no detectable algal nuclei, two possible explanations for the continued presence of these algal nuclear encoded proteins in the animal include: 1) the proteins are remarkably stable and do not turn over for 9 months (addressed in Obj. 1 above) and/or 2) the animals, via lateral gene transfer, have the coding capacity to synthesize the proteins and target them to the chloroplasts. As a first step to identifying these genes in the sea slug nuclear DNA, cDNAs to the corresponding algal nuclear genes were isolated and characterized. The full length precursors for PRK, Lhcp and psbO were found to contain an amino terminal signal peptide/transit peptide extension typical of chromophytes directing co-translational ER targeting prior to plastid import. As predicted, homology begins after the plastid targeting sequences. Since the plastids of chromophytes are located in a membrane compartment corresponding to the ER lumen, localization of nuclear encoded proteins to the plastid requires putative co-translational ER targeting prior to plastid import. However, the ctER is lost upon engulfment of the plastids by the sea slugs, therefore, uptake of proteins from animal encoded genes lacking the ER signal, would be presumably facilitated We are continuing to probe with other cDNAs to search for other nuclear encoded genes in the sea slugs to determine the extent of sea slug contribution to this unique symbiotic association.

Partial V. litorea cDNA's (discussed below) were used in early studies to probe Southern blots containing sea slug and algal DNA, however, none of the four nuclear-encoded genes tested (ITS, prk, vccp, petC) could be detected in the animal. Only the chloroplast-encoded control,

rbcL, was detectable in the sea slug. We have used Vaucheria ITS in the past to rule out the presence of algal nuclear DNA in the sea slugs. PetC was investigated because previous data indicated that the sea slugs maintain photosynthetic electron transport through PSII and PSI for at least 5 months following plastid acquisition. This nuclear encoded Reiske protein of the cytochrome b6/f complex is considered essential for this activity, but similar to the other nuclear encoded genes, it was not detected in sea slug DNA.

More recently, we designed primers to V. litorea prk and used them to attempt to amplify the genes from sea slug DNA and cDNA. Two copies of fragments of prk were found in the sea slug genomic DNA; each contained the nucleotide region spanning exon 1 and part of exon 2 of V. litorea prk, including the bipartite chloroplast transit peptide. However, the larger prk fragment possesses intron 1 while the smaller fragment is intronless. Both prk copies were detected in adult animals containing kleptoplasts as well as in plastid-free eggs produced by the sea slugs in culture. Very high similarities exist between the exon and intron sequences of prk in E. chlorotica animals and eggs and that of V. litorea. These data suggest that at least a portion of the V. litorea PRK gene was horizontally transferred at least once from the algal nuclear genome to Elysia, and it was a very recent event.

Using a similar approach, we amplified psbO from V. litorea cDNA and used it in PCR studies using sea slug cDNA and genomic DNA as templates. Fragments with high homology to the V. litorea psbO gene were obtained in both cases suggesting at least part of the psbO gene is present in the sea slug and that the gene is also expressed in the sea slugs.

While additional studies are needed to confirm these findings, including sequencing the flanking DNA in the sea slug, this is the first evidence in support of gene transfer having occurred between the alga and sea slug nuclear genomes.

Objective 3. One possible explanation for the unusually long period of time in which the algal chloroplasts are functionally associated with the sea slug is the presence of more genes than is typical for chloroplast genomes. We have now sequenced the Vaucheria litorea chloroplast genome completely. It is 115341 bp in length and contains two inverted repeats 4935 bp in length that divide the genome into a large single copy (LSC) region 62002 bp in length and a small single copy (SSC) region 43469 bp in length. The inverted repeats contain the 5S, 16S, and 23S rRNA, trnI(GAU) and trnA(UGC) genes only. A total of 138 protein-encoding genes and a normal complement of tRNA genes are contained in the LSC and SSC regions. Only one intron was found and this is in the trnL gene. An intron is found in this gene in cyanobacteria and its presence is clearly not a derived condition. The genome overall is very compact with relatively small spaces between genes. It contains more genes than land plant chloroplast genomes but less than red algal chloroplast genomes. The Vaucheria litorea chloroplast genome clearly does not contain enough genes to account for the long-lived maintenance of photosynthetic activity in slugs by itself. However, the presence of specific genes, such as rbcS, may be contributing factors.

New Obj. 4: During the summer of 2005 an undergraduate student in the lab succeeded for the first time in culturing the sea slugs through their entire life-cycle including demonstrating the uptake and incorporation of V. litorea chloroplasts. This success opens the door for numerous studies investigating the mechanism and regulation of chloroplast uptake by the sea slugs. Not only do we have direct control over the process, but we know the exact source of the chloroplasts and age and developmental stages of the sea slugs. We can now proceed with feeding/selectivity studies, microscopic analysis of the digestive system and chloroplast movement, studies on the timing of permanent establishment of the symbiosis and reproductive viability,etc., etc. We are also in a position to now provide animals for other researchers, teachers and hobbyists. We have taken still and video-images of the developmental process and are preparing teaching brochures, CD-ROMs and Web-site educational materials.

This system provides a unique opportunity to determine how components from two extremely divergent cells form a functional union, a process molecular data indicate occurred repeatedly over evolutionary history. On a much broader scale, this project possibly represents lateral gene transfer and endosymbiosis in action and is a fascinating teaching tool eliciting excitement and curiosity from people in a wide array of disciplines to the general public and especially children. The endosymbiotic theory and horizontal gene transfer continue to generate much interest and the opportunity to actually observe a symbiotic association in a potentially evolving situation is both rare and exciting.

Training and Development:

Education and training:

The PI developed a new course, Special Topics in Symbiosis, which was taught spring semester 2001 and Spring 2006 at the University of Maine. In the spring of 2004, the PI offered an advanced undergraduate/graduate course in Plant Biochemistry including aspects of chloroplast endosymbiosis and evolution. There were 6 graduate students and 2 undergraduates enrolled. In addition, thirteen undergraduates have carried out their senior research projects on the sea slug project in the PI's lab. Several undergraduates and high school students have also participated in the project. Graduate students working on the project have included one PhD and one MPS student who have completed their studies as well as a current MS student. Several Post-doctoral Associates have also been associated with the project. Guest lectures have been presented locally, at the University of New Hampshire, Boston University, College of the Atlantic, University of New Brunswick. International meeting presentations have been made at the International Symbiosis Society Congress in Halifax in 2003 and the FEBS Advanced Lecture Course in

Germany in 2005. Additional presentations will be made in 2006 (ISS Congress in Vienna) and FEBS conference in 2007. Images and information have been provided to school teachers and scientists in this country and internationally, as well as to granting agencies.

A collaboration has also been initiated with Dr. Kathleen Archer at Trinity College in Hartford, CT, a predominantly undergraduate institution, to begin to look at some of the behavioral properties of the sea slugs and the interaction of the sea slug digestive system with the chloroplasts during the period of incorporation. This involves several undergraduate students at Trinity.

Outreach Activities:

Several guest lectures have been presented locally and a demonstration was set up for the 55th and 56th annual Maine State Science Fair held at the Univ. of Maine in May, 2001 and 2002. The sea slugs have attracted several high school student tour groups to the lab. Sea slug specimens have also been provided to the New England aquarium with the goal of setting up an educational display and integrated into the public display area of the Aquaculture Research Center on the University of Maine campus. A popular article was written for the International Symbiosis Society newsletter; UMaine science writer, Nick Houtman, published an article for the UMaine Research publication; and a radio interview was given in Germany. A write-up on the collaboration with Ms. Cindy Langdon at Mary Hurd Elementary School in ME appeared in the ISS Symbiosis International Newsletter (Spring 2004, Issue #7). All of these activities help to increase the exposure of this research project and educate the general public on evolution and the possibility of lateral gene transfer between a plant and an animal resulting in the gain of photosynthetic properties by organisms which normally cannot carry out this process.

More recently, with a grant from the American Society of Plant Biologists for 2005-2006, we are developing interactive educational materials to be made available online and through CD-ROM. We will be working directly with more public school teachers to integrate these organisms and subject material into their course curricula

Journal Publications

Green B.J., W-y Li, J.R. Manhart, T.C. Fox, E.J. Summer, R.A. Kennedy, S.K. Pierce and M.E. Rumpho, "Mollusc-algal chloroplast endosymbiosis: photosynthesis, thylakoid protein maintenance, and chloroplast gene expression continue for many months in the absence of the algal nucleus.", Plant Physiology, p. 331, vol. 124, (00). Published,

Rumpho, M.E., E. Summer, B. Green, T. Fox and J. Manhart, "Mollusc/algal chloroplast symbiosis: How can isolated chloroplasts continue to function for months in the cytosol of a sea slug in the absence of an algal nucleus?", Zoology, p. 303, vol. 104, (2001). Published,

Green BJ, TC Fox, JR Manhart and ME Rumpho, "Stability of isolated chromophytic algal chloroplasts that participate in a unique molluscan/algal endosymbiosis", Symbiosis, p. 31, vol. 40, (2005). Published,

Books or Other One-time Publications

Green, Brian J., "Molecular and Biochemical Characterization of a Mollusc/Algal Chloroplast Endosymbiosis", (2000). Thesis, Published
Bibliography: PhD Thesis Texas A&M University

Rumpho, Mary E. and James R. Manhart, "Elysia chlorotica and Vaucheria litorea: Symbiosis between a mollusc and algal plastids produces solar-powered sea slugs.", (2001). Symbiosis International Publication, Published
Editor(s): Dr. Douglas Zook
Collection: Research Newsletter of the Inernational Symbiosis Society
Bibliography: Vol. 1, No. 2, Fall, 2001

Rumpho, M.E., B.J. Green, T.C. Fox and J.R. Manhart, "Unusual chloroplast stability contributes to the long-term symbiotic association between a marine mollusc and algal chloroplasts.", (2001). Online Abstract -American Society of Plant BiologistÆs Annual meeting, Published
Bibliography: Online Abstract
http://www.rycomusa.com/aspp2001/public/P22/0378.html

Rumpho, M.E., E.J. Summer and J.R. Manhart, "Mollusc/algal chloroplast symbiosis: genetic autonomy and chloroplast protein stability.", (2001). Online Abstract-Amer. Soc. Plant Biol. Mtg, Published
Bibliography: Online abstract
http://www.rycomusa.com/aspp2001/public/P22/0628.html

Lee, J, JR Manhart, ME Rumpho, "The Vaucheria chloroplast genome is less derived than green plant cpDNAs in an evolutionary context.", (2002). Electronic Abstract, Published
Bibliography: American Society of Plant BiologistÆs Annual meeting, Denver, CO (#12001)
http://abstracts.aspb.org/pb2002/public/M02/1052.html

Manhart, JR, ME Rumpho, and J Lee, "Does the chloroplast genome of V. litorea contain all the genes necessary to maintain photosynthesis in kleptoplastic sea slugs?", (2002). Electronic Abstract, Published
Bibliography: American Society of Plant BiologistÆs Annual meeting, Denver, CO (#20)http://abstracts.aspb.org/pb2002/public/P26/0272.html

Rumpho ME,Farahad P. Dastoor, Sirisha Pochareddy, Chen Cheng, Dane N. Refsland, Jungho Lee, and James R. Manhart, "Does the chloroplast genome of Vaucheria litorea contain all the genes necessary to sustain kleptoplast activity in sea slugs or has lateral gene transfer occurred?", (2003). Abstract book, Published
Editor(s): International Symbiosis Society
Collection: The 4th International Symbiosis Congress Abstract Book
Bibliography: 4th International Symbiosis Society Congress; http://people.bu.edu/iss/

Farahad P. Dastoor, Sirisha Pochareddy, Jungho Lee, James R. Manhart and Mary E. Rumpho
, "Chloroplast Protein Stability vs. De Novo Synthesis in a Sea Slug/Algal Chloroplast Kleptoplasty
", (2003). Abstract book, Published
Editor(s): International Symbiosis Society
Collection: The 4th International Symbiosis Congress Abstract Book
Bibliography: 4th International Symbiosis Society Congress; http://people.bu.edu/iss/

Rumpho ME, JR Manhart, FP Dastoor and J Lee, "The Kleptoplast.", (2006). Book, Accepted
Editor(s): RR Wise and JK Hoober
Collection: Advances in Photosynthesis and Respiration: The Structure and Function of Plastids.
Bibliography: Springer Pub., Vol. 23

Pochareddy, S, "Two Algal Phosphoribulokinase Gene Fragments Detected in the Kleptoplastic Sea Slug Elysia chlorotica", (2005). Thesis, Published
Bibliography: University of Maine, MPS Thesis

Web/Internet Site

URL(s):
http://www.umaine.edu/bmmb/faculty/rumpho.htm
Description:
This site describes my research on symbiosis and used in large part to attract prospective graduate students.

Other Specific Products

Product Type:
Data or databases
Product Description:
We have deposited gene sequences for phosphoribulokinase (AF336986), light harvesting protein complex I (AF336982), Reiske FeS protein

(AF336983), cyclophilin (AF336984) and light harvesting protein complex II (AF336985) all from Vaucheria litorea in GenBank. We have also deposted a partial sequence for actin (AF448493) from Elysia chlorotica.

We have also created a database for all the genes sequenced to date in the Vaucheria litorea chloroplast genome.

Sharing Information:

The gene sequences have been deposited in GenBank and the chloroplast genome database will be submitted for publication shortly.

Product Type:

Physical collection (samples, etc.)

Product Description:

We have the alga, Vaucheria litorea in culture and specimens of sea slugs in culture.

Sharing Information:

We share both algal and sea slug samples with interested parties as our sample size allows. Most recently we have provided sea slugs to the New England Aquarium to help establish a display there.

Product Type:

Teaching aids

Product Description:

Images of both the sea slugs and algae are provided as slides, PowerPoint presentations, overheads, and reprints as requested.

Sharing Information:

Several Email requests are received each year for images of the sea slugs to be used for teaching or for advertisement purposes to encourage students to go into science. All requests are handled promptly and images sent out according to the format preferred by the requester.

Product Type:

Audio or video products

Product Description:

In collaboration with Dr. Douglas Zook and the facilities and support of Boston University program, Preparing Tomorrow's Teachers to Teach Biology, I participated in a studio interview discussing our pioneering work with Elysia chlorotica and the plastids of Vaucheria litorea.

Sharing Information:

The teaching video is available to all online at http://people.bu.edu/iss/

Product Type:

Data or databases

Product Description:

GenBank Submission: Rumpho ME, S Pochareddy and F Dastoor 2006 Vaucheria litorea phosphoribulokinase (prk) gene, complete cds, nuclear gene for chloroplast product. DQ388997.

Sharing Information:

Public information in GenBank.

Contributions

Contributions within Discipline:

Imagine being able to turn on solar-powered cells whenever food became limiting in your environment to exploit the sun's energy to produce chemical energy. Further imagine the advantage of being mobile and camouflaged with a green, rippling leaf appearance in a sea filled with predators in search of soft-bodied creatures. The sacoglossan mollusc Elysia chlorotica (Gould) possesses all of these traits. It is a shell-less, green 'walking leaf' that will feed on algae when they are available, stealing the chloroplasts, and using them for solar-power when food is scarce. The solar-powered green cells are a result of an intracellular endosymbiosis which E. chlorotica establishes with chloroplasts from the filamentous, chromophytic alga Vaucheria litorea (C. Agardh). If provided only with light and a source of CO2, the sea slugs will survive at least 10 months in culture in the absence of any additional algae; the same life span as observed in nature where they continue to feed on algae.

The symbiotic system studied here provides a unique opportunity to determine how genetic and biochemical components from two extremely divergent organisms can form a functional and productive photosynthetic union. It is also provides the first opportunity to investigate the entire photosynthetic process in plastids separated from the influence of the rest of the cell and thus, the organism. Successful completion of the

proposed project exploiting this unusual organism should provide information on the regulatory interactions between chloroplasts and the nucleo-cytosol, the genetic autonomy of these plastids, and the mechanisms which limit chloroplast metabolism and more specifically, photosynthetic efficiency and productivity in higher plants. On a much broader scale, this project possibly represents endosymbiosis and evolution in action. The endosymbiotic theory has and continues to generate much interest at all levels, but to actually observe a symbiotic association in a potentially evolving situation and imparting such a major new function to the other partner, is indeed rare and a unique opportunity for study.

Contributions to Other Disciplines:

The results of this research would be highly beneficial to industries/growers involved in any aspect of plant/algal biomass production including: forestry, potato and blueberry production, and marine and fresh water biology. It also has applications related to global climate change and plant productivity. Furthermore, although not proposed for study here, research on the biosynthesis of secondary compounds by these organisms has implications for the medical field, particularly, in the production and identification of novel anti-cancer drugs.

The ability to now culture the molluscs through their entire life-cycle in the lab will contribute to studies on the developmental biology of this organism as well as to cell biology related to the mechanisms of uptake and incorporation of the chloroplasts in an animal. Increasing the availability of the sea slugs will contribute to other researchers interested in studying this animal as well as teachers at all levels, individual marine aquaria hobbyists, and possibly public and private aquariums and museums.

Contributions to Human Resource Development:

Thirteen undergraduates (6 males and 7 females) have carried out their senior research projects on the sea slug project in the PIs lab. Two juniors (female) and one sophomore(female) are also conducting research on the project along with an MS student (male), and a Post-doctoral Associate (male). Guest lectures have been presented locally, at the University of New Hampshire, Boston University, College of the Atlantic, and the Univ. of New Brunswick. The PI and students present annually at the American Society of Plant Biologist's meeting. The PI and students presented internationally at the International Symbiosis Society Congress in Halifax in 2003 (and will again in 2006 in Vienna) and the FEBS Advanced Lecture Course in Germany in 2005 on the 'Origin and Evolution of Mitochondria and Chloroplasts.' In addition, images and information have been provided to school teachers and scientists in this country and internationally, as well as to granting agencies.

A collaboration has been initiated with Dr. Kathleen Archer at Trinity College in Hartford, CT, a predominantly undergraduate institution, to begin to look at some of the behavioral properties of the sea slugs and the interaction of the sea slug digestive system with the chloroplasts during the period of incorporation. This involves several undergraduate students at Trinity.

Several guest lectures were presented locally and a demonstration was set up for the 56th annual Maine State Science Fair held at the Univ. of Maine in May, 2002. The sea slugs have attracted several elementary, junior and high school student tour groups to the lab and they have been adopted as the official school 'pet' at a rural Maine Elementary School, Noble VI in Berwick, Maine. Sea slug specimens have also been provided to the New England aquarium with the goal of setting up an educational display.

Contributions to Resources for Research and Education:

Through a grant from the American Society of Plant Biologists, we are developing interactive educational materials for delivery by Web and CD-ROM and working with public school teachers to incorporate the sea slugs into their course curricula in biology.

Through collaboration with Cindy Langdon at Noble VI School in Berwick,ME, we have set up new facilities for the sea slugs to be housed and on display in an elementary school. In addition, Ms. Langdon, will be setting up a Web cam to feed video of the sea slugs to the students in real-time.

Contributions Beyond Science and Engineering:

By better understanding the genetic make-up of the symbiotic chloroplasts and their regulation, we will gain a better understanding of the control of chloroplast activity by the nucleo-cytosol. This ultimately, could lead to development of drugs that specifically target chloroplast activities, for example, in the control of malaria. Furthermore, understanding the biochemistry of secondary metabolites in the sea slugs/algal chloroplast symbiosis may lead to the identification of natural anti-cancer, anti-viral compounds as has been found for other marine organisms, and sea slugs in particular. These areas of research are in progress at this time.

The ability to now culture sea slugs in the lab and make them available to other scientists and teachers will help preserve and protect a natural species. We are also studying and developing techniques novel to the aquaculture biotechnology industry, which have potential applicability to other organisms.

Conference Proceedings

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