THE 2nd INTERNATIONAL VOLVOX CONFERENCE

July 31st – August 3rd, 2013

University of New Brunswick, Fredericton
New Brunswick, Canada

SCOPE

This is the second (http://www2.unb.ca/vip/IVC2013/) of what we hope to be a long series of *Volvox* meetings to be held every other year. The idea of a meeting on everything about *Volvox* and its relatives (aka Volvocales or volvocine algae) reflects both an increase in the size of the *Volvox* community and the realization that many researchers from fields traditionally not associated with *Volvox* research (e.g., physics, theoretical biology) are interested in various aspects of the system. Indeed, volvocine algae have become an important model system for the evolution of multicellularity, development and cellular differentiation, and lately have yielded important results in fields as diverse as genomics, hydrodynamics, and social evolution. We hope that these meetings will continue to foster exchange of ideas and expertise, and will initiate new collaborations. Furthermore, with these meetings we wish to attract new people and to build a stronger *Volvox* community.

CONFERENCE ORGANIZER

Aurora M. Nedelcu, University of New Brunswick, Canada

ORGANIZING COMMITTEE

Matthew Herron, University of Montana, USA
Erik Hanschen, University of Arizona, USA
David Smith, University of Western Ontario, Canada
Hisayoshi Nozaki, University of Tokyo, Japan
James Umen, Donald Danforth Plant Science Center, USA
Stephen Miller, University of Maryland Baltimore County, USA
Annette Coleman, Brown University USA
Aurelia Honerkamp-Smith, University of Cambridge, UK

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PROGRAM AT A GLANCE

Day	Time	Activity	Location
July 31st, Wednesday			
	5:00 - 9:30	Registration	Wu Center; Foyer
	6:30 - 10:00	Welcome Reception	Wu Center; Foyer
August 1st, Thursday			
	7:30 – 8:30	Breakfast	Wu Ctr; Chancellor's Room
	8:30 – 10:00	Morning Session 1: Life Cycle	Wu Ctr; Chancellor's Room
	10:00 – 10:30	Break	Wu Ctr; Chancellor's Room
	10:30 – 12:00	Morning Session 2: Development	Wu Ctr; Chancellor's Room
	12:00 – 1:30	Lunch	Wu Ctr; Chancellor's Room
	1:30 – 3:00	Afternoon Session 1: Taxonomy	Wu Ctr; Chancellor's Room
	3:00 – 3:30	Break	Wu Ctr; Chancellor's Room
	3:30 – 5:00	Afternoon Session 2: Genetics	Wu Ctr; Chancellor's Room
	5:00 – 6:30	Poster Session	Wu Ctr; Foyer
	6:30 – 8:30	Maritime dinner	Wu Ctr; Chancellor's Room
	9:00 – 10:30	Social mixing	Dolan's Pub; Lunar Rogue
August 2nd, Friday			
	7:30 – 8:30	Breakfast	Wu Ctr; Chancellor's Room
	8:30 – 10:00	Morning Session 1: Evolution 1	Wu Ctr; Chancellor's Room
	10:00 – 10:30	Break	Wu Ctr; Chancellor's Room
	10:30 – 12:10	Morning Session 2: Evolution 2	Wu Ctr; Chancellor's Room
	12:10 – 1:30	Lunch	Wu Ctr; Chancellor's Room
	1:30 – 3:00	Afternoon Session 1: Genomics	Wu Ctr; Chancellor's Room
	3:00 – 3:30	Break	Wu Ctr; Chancellor's Room
	3:30 – 4:30	Afternoon Session 2: Workshop	Wu Ctr; Chancellor's Room
	4:30 – 5:30	Round Table	Wu Ctr; Chancellor's Room
	5:30 – 6:30	Movie and Trivia Night	Wu Ctr; Chancellor's Room
	6:30 – 10:00	Banquet - Dinner by the River	Delta Hotel
August 3rd, Saturday			
	8:30 – 10:30	Breakfast at Farmers Market	Farmers Market
	11:00 – 2:00	Boat River Cruise and Lunch	Regent Street Wharf
	2:00 -	Downtown (on your own)	Queen Str. and King Str.

SCIENTIFIC PROGRAM

THURSDAY, AUGUST 1, 2013

MORNING SESSION 1: LIFE CYCLE (Chair: Annette Coleman)

8:30 – 8:45 **Introduction**

8:45 – 9:10 **Kaoru Kawafune** (University of Tokyo)
Different rickettsial bacteria invading *Volvox carteri* by endosymbiosis and horizontal gene transfer

9:10 – 9:35 **Ben Rosenzweig** (University of Montana)
Can algae acquire Vitamin B12 from bacterial endosymbionts?

9:35 – 10:00 **Stephen Miller** (University of Maryland Baltimore County)
Effect of resource limitation on asexual development of *Volvox carteri*

MORNING SESSION 2: DEVELOPMENT & CELL DIFFERENTIATION (Chair: Stephen Miller)

10:30 – 10:45 **Introduction**

10:45 – 11:10 **Stephanie Hoehn** (University of Cambridge)
Type B embryo inversion in *Volvox globator*

11:10 – 11:35 **Patrick J. Ferris** (University of Arizona)
Evolution of a soma-determining gene

11:35 – 12:00 **Zach Grochau-Wright** (University of Arizona)
Discovery of *regA* family genes in non-*Volvox* species

AFTERNOON SESSION 1: TAXONOMY AND PHYLOGENY (Chair: Hisayoshi Nozaki)

1:30 – 1:45 **Introduction**

1:45 – 2:10 **Annette Coleman** (Brown University)
A Volvocacean jackpot

2:10 – 2:35 **Hisayoshi Nozaki** (University of Tokyo)
Two species of a new 'missing link' genus of the "evolutionary time machine" Volvocine greens

2:35 – 3:00 **Thomas Pröeschold** (University of Rostock)
How to use ITS-2 as DNA barcode marker? A case study of the Volvocales

AFTERNOON SESSION 2: MOLECULAR AND EVOLUTIONARY GENETICS (Chair: Jim Umen)

3:30 – 3:45 **Introduction**

3:45 – 4:10 **Sa Geng** (Donald Danforth Plant Science Center)
Genetic basis of sexual dimorphism in *Volvox carteri*

4:10 – 4:35 **Takako Kato-Minoura** (Chuo University)
Molecular evolution of volvocalean actin genes

4:35 – 5:00 **Aurora Nedelcu** (University of New Brunswick)
The SAND domain and the evolution of multicellular complexity

EVENING (5:00 – 6:30): POSTER SESSION

P1: Aurelia R. Honerkamp-Smith (University of Cambridge)
Selective plane illumination for 3-dimensional cell tracking during *Volvox* development and inversion

P2: Hiroko Kawai-Toyooka (University of Tokyo)
Mating type-specific two-step regulation for the fusogen GCS1 in the isogamous volvocine alga *Gonium pectorale*

P3: Arash Kianianmomeni (University of Bielefeld)
Validation of reference genes for quantitative gene expression studies in *Volvox carteri* using real-time RT-PCR

P4: Aleatha Lee (University of New Brunswick)
Volvox carteri as a model-system for cancer research

P5: Gavriel Matt (Donald Danforth Plant Science Center)
Separation of gonidia and somatic cell types for high-throughput transcriptome analysis using RNA-seq

P6: Jassy Meng (University of New Brunswick)
Volvox as a model-system for aging research

P7: Linna Meng (University of New Brunswick)
Single-domain TAZ-containing proteins potentially involved in the evolution of multicellularity in *Volvox carteri*

P8: Ayano Miyagi (Donald Danforth Plant Science Center)
Functional studies of volvocine algal *Mid* proteins in *Chlamydomonas reinhardtii*

P9: Noriko Ueki (Chuo University)
A uniquely tagged transposon for improved transposon-based mutagenesis in *Volvox carteri*

P10: Stephanie Hoehn (Cambridge University)
Biomechanical features of diverse inversion processes in *Volvox sp.*

FRIDAY, AUGUST 2, 2013

MORNING SESSION 1: EVOLUTION 1 (Chair: Matthew Herron)

8:30 – 8:45 **Introduction**

8:45 – 9:10 **Matthew Herron** (University of Montana)
Experimental evolution of a multicellular life cycle in *Chlamydomonas reinhardtii*

9:10 – 9:35 **Yoko Arakaki** (University of Tokyo)
The simplest multicellular organism *Tetrabaena socialis*

9:35 – 10:00 **Cristian Solari** (University of Buenos Aires)
Costs and benefits of the first steps toward multicellularity: A *Gonium pectorale* (Volvocaceae) case study

MORNING SESSION 2: EVOLUTION 2 (Chair: Aurora M. Nedelcu)

10:30 – 10:45 **Introduction**

10:45 – 11:10 **James Umen** (Donald Danforth Plant Science Center)
Evolution of sexual dimorphism in volvocine algae

11:10 – 11:35 **Erik R. Hanschen** (University of Arizona)
Sex and cell types: understanding the evolution of volvocine cell types

11:35 – 12:00 **Matheus Lima** (University of Sao Paulo)
Testing the oxidative damage theory of aging in *Volvox carteri*

AFTERNOON SESSION 1: GENOMICS (Chair: David Smith)

1:30 – 1:45 **Introduction**

1:45 – 2:10 **Takashi Hamaji** (Kyoto University)
Evolutionary dynamic features of mating loci inferred from de novo genome sequencing of *Gonium pectorale* (Volvocales, Chlorophyta)

2:10 – 2:35 **David R. Smith** (Western University)
The nonphotosynthetic volvocalean *Polytomella*: a genome discovery & mystery story

2:35 – 3:00 **Adrian Reyes-Prieto** (University of New Brunswick)
Exploring genomic consequences after the loss of photosynthesis in the colorless alga *Polytoma uvella*

AFTERNOON SESSION 2: GENOMICS AND WORKSHOP/ROUND TABLE (Chair: Brad Olson)

3:30 – 4:30 **Bradley J.S.C. Olson** (Kansas State University)
The Volvocales genome project

4:30 – 5:30 **Round Table**

1. Genome data analysis– approaches and challenges
2. Future comparative genomics possibilities from the Volvocales genomes
3. Working with comparative genomics data to generate candidate gene lists and validate them experimentally

EVENING SESSION

5:30 – 6:30 Movie and trivia night – Movies and trivia featuring volvocine algae

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ABSTRACTS

TALKS (by session)

Life Cycle (Chair: Annette Coleman)

DIFFERENT RICKETTSIAL BACTERIA INVADING VOLVOX CARTERI BY ENDOSYMBIOSIS AND HORIZONTAL GENE TRANSFER

Kaoru Kawafune[1], Yuichi Hongoh[2], Takashi Hamaji[3], Tomoaki Sakamoto[4], Tetsuya Kurata[4], Shunsuke Hirooka[5], Shin-ya Miyagishima[5] and Hisayoshi Nozaki[1]

1. Dept. of Biology, Grad. School of Science, University of Tokyo, Tokyo, Japan

2. Dept. of Biological Sciences, Grad. School of Bioscience and Biotechnology, Tokyo Institute of Technology, Tokyo, Japan

3. Dept. of Botany, Grad. School of Science, Kyoto University, Kyoto, Japan

4. Plant Global Education Project, Grad. School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan

5. Center for Frontier Research, National Institute of Genetics, Shizuoka, Japan

A bacterial endosymbiont was observed in the cytoplasm of *Volvox carteri* f. *weismannia* UTEX 2180 using transmission electron microscopy (Kochert & Olson 1970, Trans. Am. Microsc. Soc.), but it has not been identified using molecular methods. Recently we reported molecular identification of similar endosymbionts in other volvocaleans, *Pleodorina japonica* and *Carteria cerasiformis*, and demonstrated that the endosymbionts belong to the eubacteria family Rickettsiaceae, which consists of a major group hosted by arthropods, and "hydra group" by non-arthropods (Kawafune et al. 2012, PLoS ONE). In this study we examined the endosymbiont of *V. carteri* UTEX 2180 in a phylogenetic and fluorescence *in situ* hybridization analysis of the 16S *r*RNA gene. It belongs to the hydra group with the endosymbionts of *C. cerasiformis* and *P. japonica*. In contrast, rickettsial endosymbionts were not detected in seven other strains of three forms of *V. carteri* by DAPI-staining and genomic PCR. Nevertheless, sequences closely related to rickettsial genes were identified in the genome data of *V. carteri* f. *nagariensis* EVE10, one of the seven strains lacking the endosymbionts. Genomic PCR revealed that all seven strains possess sequences similar to rickettsial *murB*, *ddlB* and/or 16S *r*RNA genes. Phylogenetic analyses using sequences from our ongoing genome project of the *C. cerasiformis* rickettsial endosymbiont indicated that the rickettsia-like sequences from three strains of *V. carteri* f. *nagariensis* are more closely related to that of *P. japonica* than that of *V. carteri* UTEX 2180, suggesting rickettsial horizontal gene transfer independent of the rickettsial endosymbiosis in *V. carteri* UTEX 2180.

CAN ALGAE ACQUIRE VITAMIN B_{12} FROM BACTERIAL ENDOSYMBIONTS?

Ben Rosenzweig[1], Jonathan Stone[1], Matthew Herron[1]
1. Division of Biological Sciences, University of Montana, Missoula, MT, USA

Half of all known Volvocales require exogenous vitamin B_{12} (cobalamin) for growth, utilizing it primarily as a cofactor in B_{12}-dependent methionine synthesis (Croft et al. 2006, *Euk. Cell* 5:1175). Exosymbiotic bacteria in algal cultures have been identified as a potential source for the vitamin (Croft et al. 2005, *Nature* 438:90). There is evidence that this is a mutualistic relationship, with algae providing fixed carbon to bacterial co-cultures (Kazamia et al. 2012, *Env. Micro.* 14:1466). If endosymbiotic bacteria can be identified which likewise provide vitamins to their algal hosts, this would afford a unique opportunity to understand the process of endosymbiotic integration and organellogenesis. We investigate this possibility by examining the genomes of two species known to harbor rickettsial endosymbionts, *Carteria cerasiformis* and *Pleodorina japonica* (Kawafune et al. 2012, *PloS One* 7:e31749), for pseudogenization of the B_{12}-dependent methionine synthesis pathway. We also attempt to cure *C. cerasiformis* and *P. japonica* of *Rickettsia* and compare their growth on B_{12}-replete and B_{12}-deficient media.

EFFECT OF RESOURCE LIMITATION ON ASEXUAL DEVELOPMENT OF VOLVOX CARTERI

Alexandra Harryman[1], Jose Ortega Escalante[1], Jacob Kott[1], Michael Ishak[1], and Stephen M. Miller[1]
1. Department of Biological Science, University of Maryland, Baltimore County, Baltimore, MD, USA

While the effects of nutrient and light limitation on growth and development have been well studied in some green species, they are not well documented for the multicellular green alga *Volvox carteri*, despite the fact that it has long been hypothesized that the evolution of developmental complexity in this and related species was strongly influenced by ecological factors such as resource availability. Here we report findings from two studies that bear on this topic. First we documented the response of *V. carteri* to deprivation for light and two key micronutrients, phosphorus and sulfur, to determine how and when growth and development are affected by these environmental perturbations. The effects of light deprivation were obvious within 24 hours, while development of individuals deprived of phosphate and/or sulfate proceeded fairly normally for nearly two generations and did not terminate until the juvenile stage of the third generation. In no case was cell fate affected. Second, we analyzed a newly isolated, spontaneous Reg mutant derived from EVE that we named "partial Reg" ("pReg"). Under optimal growth conditions at low density, nearly all somatic cells of pReg develop normally, though in some spheroids one or a few somatic cells dedifferentiate then enlarge and develop as gonidia. However, the Reg phenotype of pReg is significantly enhanced in dense culture conditions and when pReg is grown with limiting amounts of micronutrients, including phosphorus and sulfur. These observations suggest that while

normally resource limitation has minimal effect on cell fate, there might be a cryptic pathway in *V. carteri* through which resource availability can alter somatic cell fate.

Development and Cell Differentiation (Chair: Stephen Miller)

TYPE B EMBRYO INVERSION IN VOLVOX GLOBATOR

S. Hoehn[1], A. Hallmann[2]
1. University of Cambridge, Department for Applied Mathematics and Theoretical Physics, Cambridge, UK, S.Hoehn@damtp.cam.ac.uk
2. Bielefeld University, Department for Developmental and Cell Biology of Plants, Bielefeld, DE

Spheroidal green algae of the genus *Volvox* are uniquely suited as model systems for studying the basic principles of cell sheet folding. *Volvox* embryos begin life inside out and have to invert their spherical cell-monolayer to achieve their adult configuration. The inversion process is driven by concerted changes in cellular shapes in conjunction with concerted migration of cells relative to a system of cytoplasmic bridges (CBs) [1, 2]. However, there are different sequences of inversion processes in Volvocaceae [3]. Most Studies in the recent decades focused on the type A inversion in *V. carteri*, but not much is known about inversion in other *Volvox* species. We investigated the type B inversion of *V. globator* embryos and concentrated on cell shape changes and changes in the localization of the CBs. Isolated intact, sectioned and fragmented embryos were analyzed using light microscopy, confocal laser scanning microscopy, scanning electron microscopy and transmission electron microscopy techniques. Our results show that, despite similarities between type A and type B inverters, differences exist in almost all details of the inversion processes [4]. These differences concern the occurrence of specific cell shapes as well as the spatial and temporal distribution of cell shape changes and cell movement. Based on our results and due to the cellular biomechanical implications of the involved tensile and compressive forces, we developed a global mechanistic scenario that predicts cell sheet folding during embryonic inversion in *V. globator*.

[1] Viamontes GI, Kirk DL. *J Cell Biol* 1977, 75:719-730.
[2] Hallmann A. *Protist* 2006, 157:445-461.
[3] Nishii I, Ogihara S, Kirk DL. *Cell* 2003, 113:743-753.
[4] Hoehn S, Hallmann, A. *BMC Biology* 2011, 9: 89.

EVOLUTION OF A SOMA-DETERMINING GENE

Ferris PJ[1], Hanschen ER[1], Li Q, Grochau-Wright Z[1], and Michod RE[1]
1. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson

The *regA* mutant of *Volvox carteri* has a spectacular effect on soma determination: the apparently somatic cells of the mutant spheroids soon regenerate into reproductive cells instead of undergoing cell death. *regA*, a member of the VARL (SAND) gene family,

regulates chloroplast metabolism, thereby ensuring somatic differentiation. The phylogenetic tree of all the predicted VARL domain genes in *Chlamydomonas* and *Volvox* shows that *regA* is part of a tandem array of four paralogs whose only close *Chlamydomonas* relative is *RLS1*.The genus *Volvox* is apparently polyphyletic, spread among three clades in chlpDNA gene phylogenies. *V. carteri* is included in the largest *Volvox* clade; *Volvox gigas* and *Volvox powersii* are included within a *Eudorina* clade; and the EuVolvox form a sister clade with all the anisogamous species. Previous studies predicted that *Volvox* germ-soma evolved at least twice, so the evolutionary history of *regA* remains unknown. Cosmid libraries were prepared from *V. gigas* and *Volvox ferrisii* (EuVolvox) genomic DNA, and probed with partial VARL gene sequences identified by gPCR using degenerate VARL domain primers. One cosmid from *V. gigas* contains *rlsA*, *regA* and *rlsB*; *rlsC* is on a separate cosmid. In *V. ferrisii*, an 80 kb contig generated from overlapping cosmids contains six VARL genes: *rlsD*, and five VARL genes that may correspond to the four paralogs in *V. carteri*, plus an additional paralog. Five non-VARL genes syntenic with *regA* or *rlsD* in *V. carteri* are also present in the contig. Several hypotheses exploring what early evolution of the *regA* cluster implies are discussed.

DISCOVERY OF REGA FAMILY GENES IN NON-VOLVOX SPECIES

Zachariah I. Grochau-Wright[1], Patrick J. Ferris[1], and Rick E. Michod[1]
1. Ecology and Evolutionary Biology, University of Arizona

A major goal of evolutionary biology is understanding the genetic basis of evolutionary transitions in individuality (ETI), in which a new hierarchical individual arises from the integration of lower level units into a new higher level individual. The hallmark of the ETI from unicellularity to multicellularity is the evolution of germ-soma differentiation. This is because somatic cells give up their reproductive ability for the betterment of the group and germ cells lose the ability to survive independently. *regA*, a transcription factor gene that regulates chloroplast biogenesis, is responsible for somatic differentiation in the multicellular algae *Volvox carteri*. It is a member of the VARL gene family which is expanded in V. carteri compared to *Chlamydomonas reinhardtii*, a closely related unicellular species. Previous work has shown that *regA* is part of a tandem duplication of four VARL genes known as the *regA* gene cluster. Recently, the *regA* gene cluster has been shown to be present in *Volvox gigas* and *Volvox ferrisii* as well, suggesting that the *regA* gene cluster is ancestral to many species both with and without soma. To test this hypothesis we sequenced members of the *regA* gene cluster from two species of the Volvocine algae that lack complete cellular differentiation: *Pleodorina californica* and *Eudorina elegans* var. *carteri*. Our results show that the genetic toolkit for cellular differentiation is likely present in species that lack the trait suggesting that further genetic change is required for the evolution of soma or that soma was lost multiple times.

Taxonomy and Phylogeny (Chair: Hisayoshi Nozaki)

A VOLVOCACEAN JACKPOT

AW Coleman[1]
1. BioMed, Brown University, Providence, RI 02912

A 2009 sighting of *Platydorina* in Shenzhen Reservoir, S. China, raised hopes of finally isolating and sequencing this rare alga from a non-US source. Eventually, eight samples of mud from around the edge of the reservoir were obtained. When subsamples were put out to germinate zygotes, no *Platydorina* was seen. However, the samples yielded a volvocacean jackpot; a mating pair of Gonium pectoral, two different mating pairs of *Pandorina morum*, a mating pair of *Yamagishiella*, and a mating pair of *Eudorina* of the *Platydorina*-related subclade. Also isolated were a female *Volvox carteri* and two homothallic *Eudorina/Pleodorina* clones, one dioecious and (almost) requiring acetate and the other probably monoecious and retaining the zygotes *in situ*. This rivals the famous Lemon Cove, California, site in volvocacean abundance and reinforces the notion that eutrophic reservoirs and drainage sites for broad areas tend to collect zygotes of Volvocaceae.

TWO SPECIES OF A NEW 'MISSING LINK' GENUS OF THE "EVOLUTIONARY TIME MACHINE" VOLVOCINE GREENS

Hisayoshi Nozaki[1]*, Toshihiro K. Yamada*[1]*, Fumio Takahashi*[2]*, Ryo Matsuzaki*[1] *and Takashi Nakada*[3]
1. Department of Biological Sciences, Graduate School of Science, University of Tokyo
2. Department of Biotechnology, College of Life Sciences, Ritsumeikan University
3. Systems Biology Program, Graduate School of Media and Governance, Keio University

The volvocine greens represent "an evolutionary time machine" to study origin of female-male gender and multicellularity because they encompass the entire evolutionary ranges of reproductive and vegetative morphologies (Hiraide *et al.* 2013, Mol. Biol. Evol. May issue cover). This group contains an enigmatic genus *Platydorina* with flattened vegetative colonies developing via unique embryogenesis "intercalation." However, the evolutionary scenario of origin of *Platydorina* has remained unresolved possibly because of lack of their robust sister lineage to result in ambiguous phylogenetic resolutions even using multiple chloroplast genes (e. g. Nozaki 2003; Herron *et al.* 2009, PNAS). Here we described a new colonial volvocine genus, which may represent a missing link between *Platydorina* and the typical spheroidal volvocine algae. Our chloroplast multigene phylogeny resolved that the new genus is robustly sister to *Platydorina*. This new genus has 16- or 32-celled spheroidal colonies that are similar to those of the volvocine genera *Yamagishiella* and *Eudorina*. However, it can be clearly distinguished from *Yamagishiella* and *Eudorina* by its two or three contractile vacuoles only distributed in the anterior portion of each vegetative cell and possible anisogamous sexual reproduction, although these two features are similar to those of

its sister genus *Platydorina*. Albeit rare occurrence, two species were found from a Japanese lake, and one of the two possibly grows in an Austrian freshwater.

HOW TO USE ITS-2 AS DNA BARCODE MARKER? A CASE STUDY OF THE VOLVOCALES

Thomas Pröschold[1] and Annette W. Coleman[2]
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2. Brown University, Dept. Biology and Medicine, Providence RI-02912, USA

DNA sequences are a powerful tool in systematics and molecular phylogeny of protists and have given new insights into the evolution of this group of organism. However, it has not yet proven as rewarding for taxonomic categorization. DNA Barcoding might close this gap. The goal of the International Barcoding Initiative is to find a single, universal, short DNA fragment, which is easy to sequence and leads to a clear species identification. The mitochondrial cytochrome oxidase subunit I (coxI) was proposed by the barcoding initiative and is mostly used by zoologists. However, for certain groups such as the Volvocales coxI is too conserved to separate organism at the species level. Therefore, several other markers such as rbcL and matK have been proposed as suitable barcodes. But there is a conflict, and the search for best barcode marker and DNA Barcoding in general are still under controversial discussion. Especially the high number of submitted barcodes showing that the biodiversity is much higher than expected is in contrast to the limits of traditional taxonomy, which requires long term investigations and experience. In addition, especially for microalgae and protists the question about which species concept should be used is still not answered satisfactorily. To solve these conflicts, we used in our study the second Internal Transcribed Spacer (ITS-2) of the nuclear ribosomal gene cistron. This locus has a high degree of predictability across eukaryotes, is easy to sequence, and its secondary structure can be used for comparison at species and generic levels, which is common practice by taxonomists. The main objection to the ITS-2 usage as barcode marker was the difficulty in aligning these sequences and the prediction of the secondary structure. However, with the help of the new computer programmes and the easy recognition of two hallmarks in the secondary structure, these problems are resolved. ITS-2 also gives additional information about the species concept. For example, compensatory base changes (CBC) in the 30 bp highly conserved region of Helix III of ITS-2 correlate with the extent of sexual compatibility. A difference of even one CBC in this region predicts a total failure of crossing. In addition the newly proposed ITS-2 barcode can be used for the identification of hidden diversity as demonstrated for *Pandorina morum* complex.

Molecular and Evolutionary Genetics (Chair: James Umen)

GENETIC BASIS OF SEXUAL DIMORPHISM IN VOLVOX CARTERI

Sa Geng[1], James G. Umen[1]
1. Donald Danforth Plant Science Center, 975 North Warson Rd. St. Louis, MO 63132

Chlamydomonas, *Volvox* and other Volvocine algae are a unique comparative model for the evolution of sex chromosomes and gender. In *Chlamydomonas* a single *MT-* gene called *crMid* (minus dominance) determines mating phenotype and its expression is induced by the absence of nitrogen. Mid orthologs are present throughout the Volvocine algal lineage in either *MT-* or male strains of each species. The *Volvox Mid* gene (*vcMid*) is found only in male *MT* and is expressed in both vegetative and sexual stages. We used two approaches to test the role of vcMid in *Volvox* sex determination: generation of female transgenics that express vcMid, and generation of male transgenics with reduced vcMid expression. We constructed female *Eve::Mid-T* lines expressing *vcMid* constitutively. These lines appear normal when grown vegetatively but when sexually induced produce ~32 large reproductive germ cell precursors that do not become eggs, but instead undergo additional cleavage divisions to produce functional sperm packets. In a complementary approach we introduced an inverted hairpin construct (*vcMid-hp*) targeting the endogenous *vcMid* gene into *Volvox* males to generate knockdown lines. *AichiM:vcMid-hp* lines appear normal when grown vegetatively, but when exposed to sex inducer show a novel phenotype: They undergo early embryonic cleavage in a pattern that is identical to wild type males to produce 128 somatic and 128 large cells; however, the large cells do not undergo further cleavage into sperm packets, but instead differentiate as eggs that can be fertilized by wild-type sperm. These findings show that Mid proteins are conserved as key sex-determining genes in Volvocine algae, but that additional regulators in *Volvox MT* are required to specify germ cell precursor patterning during embryogenesis.

MOLECULAR EVOLUTION OF VOLVOCALEAN ACTIN GENES

Takako Kato-Minoura[1], Mika Ehara[1], Kumiko Karino[1], Nobuyuki Akimoto[1], Norito Yoshiga[1], and Seishiro Aoki[2]
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The unicellular green alga *Chlamydomonas reinhardtii* has two copies of actin gene: a conventional actin (90% amino acid identity to mammalian actins) and NAP (novel actin-like protein). NAP shares only 64 % amino acid identity with conventional actins. Our previous study has identified two NAP homologs from related algae, *Chlamydomonas moewusii* and *Volvox carteri* (Kato-Minoura et al., 2003). We could not, however, infer the origin of NAP by phylogenetic analyses using these partial sequences. Here, we determined the full-length genomic sequences of *C. moewusii* NAP, *C. moewusii* actin, and *Gonium pectorale* actin, as

well as partial genomic sequences of *Volvulina steinii* NAP, *V. steinii* actin, and *Eudorina elegans* actin. Using the NAP and actin sequences from our study, previous studies, and an analysis of draft genome sequence of *G. pectorale* (courtesy of Dr. Nozaki, Univ. of Tokyo and Dr. Fujiyama, Nat. Inst. Genetics), we re-examined the phylogenetic relationship between these actin and NAP sequences. Both maximum-likelihood and Bayesian phylogenetic analyses supported the monophyly of NAP homologues of the Chlorophyta. The results also suggested that the NAP gene is a paralog of the conventional actin and originated from gene duplication in the clade of Chlorophyta. The duplication event may have occurred after the divergence of Chlorophyta from the ancestor of green plants. The branch lengths of NAP homologs were extremely long, which suggests that the nucleotide substitution rate of NAP genes is much higher than that of actin genes.

THE SAND DOMAIN AND THE EVOLUTION OF MULTICELLULAR COMPLEXITY

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Multicellularity has evolved independently in at least 25 separate lineages from all three domains of life. However, multicellular forms with differentiated cell types are only known in a handful of groups. Complex multicellular body forms require sophisticated mechanisms that control cell proliferation and differentiation, and additional levels of gene regulation (including new transcription factors and new regulatory elements) have evolved in these lineages. The SAND domain is a conserved ~80 residue region found in a number of nuclear proteins with roles in chromatin-dependent transcriptional control. Proteins containing the SAND domain are involved in the control of cell proliferation and cell differentiation in three lineages in which complex multicellularity evolved independently: land plants (*ULTRAPETALA*), *Volvox carteri* (*RegA*), and animals (*Sp100*, *DEAF*-1, *Spe44*). We have performed an extensive search for genes coding for SAND domains across the eukaryotic tree. Interestingly, the phylogenetic distribution of genes encoding SAND domains is restricted to Viridiplantae (green algae and land plants) and Metazoa. Furthermore, although SAND domains are found in complex proteins (in combination with several other types of domains), the land plant ULTRAPETALA, the *V. carteri RegA*, and the animal *Spe44*, are all simple, single-domain proteins. Here, we discuss the evolutionary history of SAND domain-containing proteins, including the possibility that Metazoa acquired the SAND domain from an algal lineage, via lateral gene transfer.

Evolution 1 (Chair: Matthew Herron)

EXPERIMENTAL EVOLUTION OF A MULTICELLULAR LIFE CYCLE IN CHLAMYDOMONAS REINHARDTII

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The transition to multicellularity made possible the evolution of large, complex organisms, but early steps in this transition remain poorly understood. The vast majority of multicellular organisms possess a complex life history that includes development from a single cell. Here we show that multicellular complexity, including development from a single cell, can rapidly evolve from a primitively unicellular ancestor. We subjected the unicellular green alga *Chlamydomonas reinhardtii* to conditions that favor the evolution of multicellularity, resulting in the evolution of a temporally-dynamic multicellular life cycle characterized by discrete phases of dispersal and growth. Shortly after transfer to fresh medium, multicellular clusters reproduce by releasing motile unicellular propagules, which subsequently lose motility and themselves develop into clusters. A single-cell bottleneck in the ontogeny of multicellular taxa is widely believed to be an adaptation to prevent selfish cell-level evolution from undermining multicellular complexity. In our system, however, we find that a single-cell bottleneck is present at the origin of multicellularity. These results demonstrate that multicellular complexity can evolve rapidly, and suggest that alternation between uni- and multicellular life stages may have been one of the first traits to evolve during the transition to multicellularity.

THE SIMPLEST MULTICELLULAR ORGANISM TETRABAENA SOCIALIS

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Volvocine green algae represent the “evolutionary time machine” model lineage for studying multicellularity. Multicellular volvocalean species including 16-celled *Gonium pectorale* and *Volvox carteri* generally have several common morphological features to survive as integrated multicellular organisms such as “rotational asymmetry of cell architecture” to let the cells become components of the individual and “cytoplasmic bridges between protoplasts in developing embryos” to maintain the species-specific form of the multicellular individual before secretion of new extracellular matrix (ECM). However, these morphological features have not been studied in the four-celled volvocine species such as *Tetrabaena socialis* that is positioned in the most basal lineage within the colonial or multicellular volvocine greens. Here we established synchronous cultures of *T. socialis* and carried out immunofluorescence microscopic and ultrastructural observations to elucidate these two morphological attributes. Based on immunofluorescence microscopy, four cells of the mature *T. socialis* colony were identical in morphology but had rotational asymmetry in arrangement of microtubular rootlets like *G. pectorale* and *V. carteri*. Ultrastructual observations clearly confirmed the

presence of cytoplasmic bridges between protoplasts in developing embryos of *T. socialis* even after the formation of new flagella in each daughter protoplast within the parental ECM. Therefore, these two morphological attributes might have evolved in the common ancestor of the colonial volvocine algae and formed the basis for the further increase in cell number and complexity of the multicellular individuals of this lineage. *T. socialis* is one of the simplest integrated multicellular organisms in which four identical cells constitute the individual.

COST AND BENEFITS OF THE FIRST STEPS TOWARD MULTICELLULARITY: A GONIUM PECTORALE (VOLVOCACEAE) CASE STUDY

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What were the size-related advantages that caused single cells to start living in groups? Primordial groups of undifferentiated cells might have benefited from decreased predation, as well as having novel opportunities to increase nutrient uptake, nutrient storage, and possibly enhance motility capabilities, to name a few of the dominant hypotheses. We have tested these hypotheses in *Gonium pectorale*, a colonial volvocine green algae that ranges from 1 to 16 cells. We have analyzed previous data of the Volvocales on motility and growth rates, and performed experiments at different nutrient concentrations and temperatures to compare growth rates between *G. pectorale* and the unicellular volvocine algae *Chlamydomonas reinhardtii*. Using the phagotrophic euglenoid *Peranema trichophorum*, we have also measured predation rates on *C. reinhardtii* and *G. pectorale* populations composed of colonies of different sizes. Our analysis supports the hypothesis that predation pressure was an important selective pressure for the origin of multicellularity. We think that the extra-cellular matrix necessary for cell clustering might have been co-opted for storage and used as a nutrient source when needed. Our evidence backed by theoretical analysis shows that increased motility and nutrient uptake were probably not selective advantages for the first cell groups.

Evolution 2 (Chair: Aurora Nedelcu)

EVOLUTION OF SEXUAL DIMORPHISM IN VOLVOCINE ALGAE

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Volvocine algae are a unique system for investigating the evolution of sex. Plants, animals and other multicellular species are mostly oogamous with small male and large female gametes, while most unicellular species are isogamous with equal-sized gametes. We employ comparative approaches and molecular genetics to understand the transition from isogamy to oogamy using the model species *Chlamydomonas reinhardtii* and *Volvox carteri*. *C. reinhardtii* is isogamous and undergoes gametogenesis in response to nitrogen deprivation (-N), whereas *V. carteri* is oogamous and undergoes dimorphic sexual development to form

eggs and sperm in response to a diffusible sex-inducer glycoprotein. How did these morphological differences and other changes in sexual development evolve among Volvocine algae and what are their genetic bases? The sexual cycle in Volvocine algae is governed by a large, multigenic and structurally dimorphic mating locus (*MT*) that controls sexual differentiation, fertilization and zygote development. Although *MT* loci from *C. reinhardtii* and *V. carteri* descended from a common chromosomal region, they differ greatly in size, structure and divergence rates between haplotypes. Our work focuses on how differences in *MT* from the two species contributed to evolution of their divergent sexual cycles. In *Chlamydomonas* the *Mid* gene is present only in the *MT*- locus and governs sexual differentiation: gametes expressing *Mid* differentiate as *minus* mating type and those that do not express *Mid* mate differentiate as *plus* mating type. *Mid* orthologs are found in *MT*- or male *MT* from all Volvocine algae examined to date. Our recent work has revealed that *V. carteri Mid* (*VcMid*) is a key regulator of germ cell differentiation but that it is insufficient to control the entire dimorphic developmental program that characterizes *V. carteri* males versus females. We are focusing on several key questions that pertain to the evolution of Mid and its role in sexual differentiation: How is VcMid activity or expression regulated in *V. carteri*? What are the structural features that distinguish CrMid and VcMid? What are the transcriptional targets and interacting partners of CrMid and VcMid? Besides *VcMid*, which other mating locus genes in *V. carteri* contribute to the sexual cycle? Our progress in answering these questions will be presented.

SEX & CELL TYPES: UNDERSTANDING THE EVOLUTION OF VOLVOCINE CELL TYPES

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One of the hallmarks of multicellularity is the division of labor via cellular differentiation. This division of labor reduces fitness at the lower level and exports fitness to the higher level, thereby ensuring individuality at the higher level. Previous research has hypothesized that cell types in multicellular species may evolve from temporally transient cell types in unicellular ancestors. Under this hypothesis, a temporal response to environmental fluctuations is co-opted into a spatial context and a multicellular organism evolves distinct cell types. This hypothesis is supported by genetic data in the volvocine algae and metazoans, but these examples rely on relatively divergent comparisons. Here, we test this hypothesis using a life history approach, comparing two similar species with different number of cell types. The volvocine algae, with species ranging from unicellular *Chlamydomonas reinhardtii*, to multicellular *Volvox carteri* (with two cell types, germ and soma), are an excellent model system for the evolution of cellular differentiation. We specifically investigate the evolution of three cell types in *Volvox rousseletii*, as compared to *V. carteri*. The third cell type in *V. rousseletii* appears to be plastic, functioning as additional somatic cells in some environments and functioning as additional germ cells in other environments. By modeling random environmental variation, we find that high rates of

environmental change and relatively low proportions of poor environmental conditions will select for additional cellular differentiation in the volvocine algae.

TESTING THE OXIDATIVE DAMAGE THEORY OF AGING IN VOLVOX CARTERI

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Although aging is a universal phenomenon, how and why aging happens is not fully understood. Various mechanistic and evolutionary theories have been proposed to explore these questions. Of the former, the oxidative damage theory (ODT) is the most studied. ODT proposes that aging is a consequence of cellular damage caused by reactive oxygen species. Most experimental work related to this theory was performed in animal model-systems (e.g., fly, nematode) and yeast. Here, we are using a photosynthetic system – the green alga, *Volvox carteri* - to further investigate the oxidative damage theory of aging. The theory posits that factors that increase oxidative damage should accelerate senescence, while factors that decrease damage should delay senescence. To address this theory we manipulated the levels of oxidative stress by altering light intensity and temperature, as well as by adding antioxidants. Under these experimental conditions, we assessed somatic cell viability, onset of aging (chronological and developmental), and rate of aging. Our data support the ODT and suggest that aging in algae is also associated with oxidative stress. More generally, this study argues for the suitability of *Volvox* as a model-system to explore other theories of aging.

Genomics (Chair: David Smith)

EVOLUTIONARY DYNAMIC FEATURES OF MATING LOCI INFERRED FROM DE NOVO GENOME SEQUENCING OF GONIUM PECTORALE (VOLVOCALES, CHLOROPHYTA)

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We investigated the molecular genetic origins of male–female sexual dimorphism based on comparisons of mating loci (*MT*) across the volvocine green algal lineage. *MT* of the oogamous multicellular *Volvox carteri* shows significant expansion, acquisition of 13 novel gender-specific genes, and extreme divergence of shared genes (alleles present in both haplotypes of *MT*) compared with that of the isogamous unicellular *Chlamydomonas reinhardtii* (Ferris et al. 2010, Science). We partially determined *MT* of the isogamous 16-celled volvocine alga *Gonium pectorale* with chromosomal walking on BAC libraries, demonstrating little divergence of shared genes and the presence of a sex-regulated *FUS1* gene homolog in the *MT*+ haplotype (Hamaji et al. 2011, IVC-1). However, origins of the novel gender-specific genes of *V. carteri* and *MT* loci itself remained unknown. Here we determined the whole genome sequences including *MT* of *G. pectorale*. The rearranged portion of *G. pectorale MT* (R-domain) encompasses at least 400 kb making it moderately larger than the R-domain of *C. reinhardtii MT* that is 200-300 kb. No homologs of gender-specific genes except for *FUS1*, *MID* and *MTD1* in *C. reinhardtii* or *V. carteri* were found in the *G. pectorale MT* or elsewhere in the *G. pectorale* genome. Synteny mapping of *G. pectorale* draft genome sequence onto *C. reinhardtii* chromosomes, and linkage mapping of *G. pectorale MT* and other scaffolds, suggest that *MT* has the same chromosomal origin (common with *C. reinhardtii* chromosome 6 or *V. carteri* linkage group I) in the volvocine lineage, despite dynamic rearrangements (insertions or deletions) of the shared genes.

THE NONPHOTOSYNTHETIC VOLVOCALEAN POLYTOMELLA: A GENOME DISCOVERY & MYSTERY STORY

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The *Polytomella* group is peculiar. Sandwiched between popular model organisms, like *Chlamydomonas reinhardtii* and *Dunaliella salina* (Smith et al. 2010), it comprises poorly studied nonphotosynthetic unicells, which bear four flagella and are found in diverse habitats, such as freshwater pools of rotting hemp. This talk highlights recent genetic insights into the *Polytomella* genus, including the discovery of bizarre mitochondrial genome architectures and the possible absence of a plastid genome. These data are compared to those of other volvocalean algae, and the potential for research community involvement in a *Polytomella* genome project are discussed.

EXPLORING GENOMIC CONSEQUENCES AFTER THE LOSS OF PHOTOSYNTHESIS IN THE COLORLESS ALGA POLYTOMA UVELLA

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The loss of photosynthesis in unicellular green algae has occurred several independent times during the evolution of the group. Here, we present a genome scale analysis of two

Chlamydomonadaceae algae: the colorless *Polytoma uvella* (Ehrenberg) and the photosynthetic *Chlamydomonas leiostraca* (Strehlow). Phylogenetic analyses of nuclear and plastid markers indicate that *P. uvella* and *C. leiostraca* are closely related taxa, providing us with an outstanding model to study the transition from autotrophic to heterotrophic metabolism in free-living algae. Specifically, our study investigated the organellar and nuclear genomic changes occurred after the loss of photoautotrophic metabolism in *P. uvella*. As expected, the results of this comparative study suggest that the plastid genome of *P. uvella* has suffered a process of reduction. We will present and discuss unambiguous evidence of plastid-derived genes present in the *P. uvella* genomic repertoire, the nature of the colorless plastid genome and the putative functions retained in the formerly photosynthetic organelle of this colorless alga.

Genomics Workshop (Chair: David Smith)

THE VOLVOCALES GENOME PROJECT

Bradley J.S.C. Olson[1], Erik R. Hanschen[2], Tara N Marriage[1], Takashi Hamaji[3,4], Pierre Durand[2], Hisayoshi Nozak[i3], Patrick J. Ferris[2] Jonathan Featherston[5], Masahiro Suzuki[3], Hiroko Kawai-Toyooka[3], Asao Fujiyama[6], Atsushi Toyoda[6], Susan Brown[1], Michelle Coleman[1], and Richard E. Michod[2]

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The Volvocales and their unicellular relatives have long been viewed as an important model system for the evolution of multicellularity and cellular differentiation. *Chlamydomonas* and to a lesser degree *Volvox* are well developed molecular-genetic model systems, but other Volvocacean species representing important evolutionary steps toward multicellularity, have not been as well developed. The completion of the genomes of *Chlamydomonas* and *Volvox* has demonstrated that even though these organisms differ markedly in the morphology, their genomes are surprisingly similar. This suggests that the evolutionary path to multicellularity and cellular differentiation requires only a few genetics changes. With the availability of next-generation sequencing technology, the Volvocales Genome Project seeks to focus initially on sequencing the genomes of *Gonium pectorale, Basichlamys sacculifera, Tetrabaena socialis, Astrephomene gubernaculifera, Pandorina morum, Volvox globator, Yamagishiella unicocca, Eudorina elegans and Pleodorina starii, Volvox aureus* and *Volvox obversus.* Currently, the project is nearing completion of the *G. pectorale* genome sequence, annotation and a corresponding life-cycle transcriptome. Analysis of the *G. pectorale* genome suggests many of the genetic differences present in the *V. carteri* genome, except the

evolution of *regA*, occurred very soon after colonial multicellularity evolved. The primary differences found in the *G. pectorale* genome compare to *C. reinhardtii* are in cell cycle regulatory genes, and in cell wall and ECM related genes.

Poster Session

P1 *SELECTIVE PLANE ILLUMINATION FOR 3-DIMENSIONAL CELL TRACKING DURING VOLVOX DEVELOPMENT AND INVERSION*

Aurelia Honerkamp-Smith[1], Stephanie Hoehn[1], Jocelyn Dunstan[1], Raymond E. Goldstein[1]
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We are constructing a Selective Plane Illumination Microscope based on the Open SPIM project (www.openspim.org) with the goal of ascertaining the location and identity of each cell in an embryo during cell division, differentiation, and inversion. This detailed information will allow us to describe the geometry changes that inverting embryos undergo *in vivo* and in real time. We will also be able to determine the lineage and geometric placement of cells during the process of cell division.

P2 *MATING TYPE-SPECIFIC TWO-STEP REGULATION FOR THE FUSOGEN GCS1 IN THE ISOGAMOUS VOLVOCINE ALGA GONIUM PECTORALE*

Hiroko Kawai-Toyooka[1], Toshiyuki Mori[2], Takashi Hamaji[3], Masahiro Suzuki[1], Bradley J.S.C. Olson[4] and Hisayoshi Nozaki[1]
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GCS1/HAP2 is a widely conserved, generally male-specific transmembrane protein essential for fertilization. In *Chlamydomonas reinhardtii,* fertilization is achieved by the fusion of two different mating-type gametes with similar size/shape but their mating structures are morphologically different; *plus* gametes bear an actin-filled tubular mating structure (TMS), whereas *minus* gametes has a much shorter mating structure without actin accumulations. GCS1/HAP2 in *C. reinhardtii* is localized at the *minus* mating structure, and mediates membrane fusion with *plus* gametes. As contrasted to *C. reinhardtii*, both mating-type gametes in *Gonium pectorale* are morphologically identical with TMS, offering a striking advantage to focus on the cell biological basis for the mating type-specific action of GCS1/HAP2. Recently, we reported the identification of a *GCS1/HAP2* orthologue from *G. pectorale* (*GpGCS1*) and its increased expression in *minus* gametes (Kawai-Toyooka *et al.*, Chlamydomonas meeting 2012). In this study, we raised an antibody against GpGCS1 and used for expression/localization analyses. GpGCS1 was more abundantly, but not exclusively, expressed in *minus* gametes compared to *plus* gametes. Before activation, GpGCS1 accumulates at the anterior end of the cells where TMS will be formed in activated *minus* gametes, while it was observed in the internal cytoplasmic region in *plus* gametes.

Upon activation, GpGCS1 was present at the surface of *minus* TMS but not of *plus* TMS. These results suggest the presence of the two-step, *minus*-specific regulatory mechanism for GpGCS1; 1) transcriptional regulation to enrich GpGCS1; 2) post-translational transportation

P3 *VALIDATION OF REFERENCE GENS FOR QUANTITATIVE GENE EXPRESSION STUDIES IN VOLVOX CARTERI USING REAL-TIME RT-PCR*

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Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) is a sensitive technique for analysis of gene expression under a wide diversity of biological conditions. However, the identification of suitable reference genes is a critical factor for analysis of gene expression data. To determine potential reference genes for normalization of qRT-PCR data in the green alga *Volvox carteri*, the transcript levels of ten candidate reference genes were measured by qRT-PCR in three experimental sample pools containing different developmental stages, cell types and stress treatments. The expression stability of the candidate reference genes was then calculated using the algorithms geNorm, NormFinder and BestKeeper. The genes for 18S ribosomal RNA (*18S*) and eukaryotic translation elongation factor 1α2 (*eef1*) turned out to have the most stable expression levels among the samples both from different developmental stages and different stress treatments. The genes for the ribosomal protein L23 (*rpl23*) and the TATA-box binding protein (*tbpA*) showed equivalent transcript levels in the comparison of different cell types, and therefore, can be used as reference genes for cell-type specific gene expression analysis. Our results indicate that more than one reference gene is required for accurate normalization of qRT-PCRs in *Volvox carteri*. The reference genes in our study show a much better performance than the housekeeping genes used as a reference in previous studies.

P4 *VOLVOX CARTERI AS A MODEL-SYSTEM FOR CANCER RESEARCH*

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Cancer is the breakdown of cooperation among the cells of a multicellular organism, and can result in the demise of the individual. Consequently, multicellular organisms evolved a variety of mechanisms to maintain cooperation and prevent uncontrolled cell proliferation. One mechanism that can favor/stabilize cooperation is antagonistic pleiotropy. Under this scenario, the cooperative gene has a pleiotropic effect on another fitness component at the individual level, such that selfish mutants will be at disadvantage in particular settings. As cancer cells can be viewed as selfish cells, we hypothesize that their short-term gain in terms of replication comes with a cost in other components of fitness in specific settings. This hypothesis predicts that there are conditions in which cancer cells would do worse than the somatic cells. To address this possibility we are using *Volvox carteri* – a simple multicellular green alga with only 2 cell types: reproductive and non-replicating terminally differentiated somatic cells. In *V. carteri*, one gene – *regA* – appears to be necessary and sufficient to

suppress immortality in the somatic cells; single mutations in this gene render the somatic cells immortal (cancer-like). In this context, *regA* can be considered a cooperation gene. We are currently using 2 *regA* mutants to assess the effect of various environmental stresses on their fitness compared to the wild-type. Our preliminary data suggest that *regA* mutants are more sensitive to changes in environmental conditions relative to the wild-type. These results provide proof of principle for developing therapeutic strategies that take advantage of the evolutionary vulnerabilities of cancer cells by exposing their weaknesses.

P5 *SEPARATION OF GONIDIA AND SOMATIC CELL TYPES FOR HIGH-THROUGHPUT TRANSCRIPTOME ANALYSIS USING RNA-SEQ*

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Volvox carteri is composed of two distinct cell types: reproductive gonidia cells and terminally differentiated somatic cells. Transcriptomic profiles of both cell types will be an important tool for understanding the regulation of this germ-soma dichotomy at the molecular level. Previous work has utilized low-throughput techniques, such as Northern blots and RT-PCR, to identify cell-type specific gene expression [1,2]. We are now undertaking a high-throughput analysis of the gonidia and somatic transcriptomes using RNA-seq. Towards this goal we have developed a modified method for rapid and efficient separation of gonidia and somatic cells that will help maintain transcriptome integrity for downstream analyses. RNA samples from these preparations have been validated with cell-type specific markers and are in process for library preparation and deep transcriptome sequencing. This work will help identify the metabolic and regulatory programs that distinguish the two cell types, will establish molecular markers specific to a germ or somatic fate, and will provide a platform to compare transcriptomes of germ-soma dichotomy mutants, such as *regA* (somatic-regenerator), *lag* (late-gonidia), and *gls* (gonidialess).
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P6 *VOLVOX AS A MODEL-SYSTEM FOR AGING RESEARCH*

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Aging – defined as the time-dependent decline in fitness that occurs after an individual reaches its maximum reproductive potential – is a universal phenomenon in multicellular organisms. Understanding how and why aging occurs has been a long-standing problem in biology. In this study we addressed several theories of aging and tested specific predictions using the multicellular green alga, *Volvox*, as a model system. Alteration of light intensity and exposure time as well as the addition of an antioxidant had a significant effect on the lifespan and onset of aging. Growth at low temperatures and low nutrients showed a

remarkable increase in lifespan but this was associated with a decrease in fitness during the reproductive phase. Differences in the onset of aging have also been observed between two species of *Volvox* and between a wild-type and a developmental mutant strain. Overall, the data reported here are consistent with the oxidative stress and the caloric restriction theories, but not with the disposable soma theory; also, the data provide support for the mutation accumulation and the by-product of development theories, and indirectly, for the antagonistic pleiotropy theory. In addition, our data show that lifespan and the onset of aging vary independently of each other, and extension of lifespan always comes with a fitness cost. Lastly, this study argues for the suitability of *Volvox* as a model system to develop a more integrative view of aging, from both a mechanistic and evolutionary perspective.

P7 *SINGLE-DOMAIN TAZ-CONTAINING PROTEINS POTENTIALLY INVOLVED IN THE EVOLUTION OF MULTICELLULARITY IN VOLVOX CARTERI*

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The volvocine algae have emerged as an effective model-system for investigating the genetic changes associated with the transition from unicellularity to multicellularity. Comparative analyses of the genomes of the unicellular *Chlamydomonas reinhardtii* and the multicellular *Volvox carteri* revealed no major differences between the two genomes, with the exception of 3 gene families that appear to have expanded in the lineage leading to *V. carteri*; these genes families encode proteins associated with cell-cycle regulation and the extracellular matrix. Another family that is larger in *V. carteri* relative to *C. reinhardtii* contains genes coding for the transcriptional co-activators CREB-binding protein (CBP) and P300, which act as histone acetyltransferases that regulate transcription via chromatin remodeling; these complex proteins contain several domains including two Zinc fingers of the TAZ type. Although both *C. reinhardtii* and *V. carteri* genomes each code for one CBP/P300 protein, the *V. carteri* genome also encodes three single TAZ domain proteins while the *C. reinhardtii* genome only has one. In animals, CBP is known to interact and enhance the activity of AIRE – a SAND domain-containing transcription factor. Interestingly, RegA – the transcription factor responsible for the differentiation of somatic cells in *V. carteri* – also contains a SAND domain. To address the possibility that TAZ-containing proteins have been involved in the evolution of multicellularity/cell differentiation in *V. carteri*, we investigated the evolutionary history of TAZ-containing proteins in both the green algal group and across the eukaryotic tree.

P8 *FUNCTIONAL STUDIES OF VOLVOCINE ALGAL MID PROTEINS IN CHLAMYDOMONAS REINHARDTII*

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Chlamydomonas, *Volvox* and other Volvocine algae are a unique comparative model for the evolution of sex chromosomes and gender. We have focused on the key sex determining

gene called MID, a putative RWP-RK transcription factor encoded by the *minus* mating type (*MT-*) in *Chlamydomonas reinhardtii* (crMid) and male *MT* (*MTM*) in *Volvox carteri* (vcMid). While *crMid* specifies minus mating type and its expression is induced by nitrogen deprivation, vcMid is expressed constitutively in males. To test if vcMid can fulfill the function of crMid we constructed HA-tagged vcMID constructs whose expression is driven by a constitutive promoter for expression in a *Chlamydomonas mid-1* mutant strain. We tested constructs that include full-length crMid and vcMid as well as chimeras with the crMid N-terminal domain and vcMid DNA-binding domain, and vice versa. However, only the complete *crMid* construct was able to complement the *mid-1* mutation. These data suggest that the N-terminal and DNA binding domains of crMid are both required for function. Interestingly, crMid-HA protein is only detectable in gametes and not in vegetative cells even though its expression is driven by a constitutive promoter. Our results indicate that crMid expression may be controlled at multiple levels including mRNA production and posttranscriptional processes. Studies of intracellular crMid localization are also under way.

P9 *A UNIQUELY TAGGED TRANSPOSON FOR IMPROVED TRANSPOSON-BASED MUTAGENESIS IN VOLVOX CARTERI*

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Transposon-based mutagenesis, or transposon tagging, is a powerful method for tagging, mutating and cloning genes responsible for a particular phenotype. In *Volvox carteri,* two endogenous transposons, *Jordan* and *Idaten*, have been used previously for gene tagging and identification of developmentally important genes. A major difficulty with this method is the large number of genomic copies of these transposons, which makes it necessary to identify the copy that caused the mutation among more than 50 copies in the genome by RFLP analysis. Here we tried to improve the transposon-tagging method by generating a *Volvox* strain that contains a modified, transposable copy of the *Idaten* transposon with a unique, sequence-tagged site (STS). A sequence alignment of two copies of *Idaten* with verified transposition capability allowed for the identification of a short segment without sequence similarity. Moreover, the other genomic copies of *Idaten* also showed most variability in this segment. Therefore, this sequence segment seemed to be non-critical for transposition. A plasmid, p*Idaten*, was constructed in which the non-critical segment of *Idaten* was replaced by a unique STS of 387 bp and then *Volvox carteri* was transformed with p*Idaten*. Many of the resulted transformants contained numerous copies of the tagged *Idaten* and frequently the tagged *Idaten* did not contain the full length, probably due to its large size of 12.6 kb. After screening by PCR and Southern blot analysis, finally three *Volvox* transformants were identified that harbor two to six copies of the complete *Idaten* with STS. If cold-induced transposition of an *Idaten* with STS causes mutation of an unknown gene, the cumbersome and tedious RFLP analysis will be shortened dramatically by using the STS as a probe.

P10 *BIOMECHANICAL FEATURES OF DIVERSE INVERSION PROCESSES IN VOLVOX SP.*

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The embryonic inversion processes in different *Volvox*-species can be categorized as type A (e.g. *V. carteri, V. gigas*) [1, 2], type B (e.g. *V. globator*) [3] or as intermediate type A/B (e.g. *V. tertius*) [4, 5]. Type A inverters possess four `lips' at their anterior pole which curl outward at the beginning of inversion. In type B-inversion a bend region appears at the equator of the embryo, the posterior hemisphere inverts and the anterior hemisphere seems to be pulled `downwards'. According to corresponding literature, the embryos of *V. tertius* do possess four lips at their anterior pole, but the bend region is formed between the anterior pole of the embryo and its equator and the four lips curl backward later than in type A-inversion [4, 5]. To elucidate the specific cell shape changes that underlie such intermediate inversion processes we are comparing inversion in different *Volvox*-species via time-lapse video microscopy and light microscopy of semithin sections.

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