SEEDS: 'You Choose' Awards

Forward Genetics, Transcriptomics, and the Early Ctenophore Embryo: The Initial Acquisition of Differential Cell Fates

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Abstract

Analyses of transcriptomes, sets of expressed RNA's, in cells of biological interest provides critical molecular genetic clues concerning the basic attributes of differentiated cell types sharing the same static genome. I propose carrying out a transcriptome based forward genetic screen to identify genes associated with the first restriction of developmental potential during Mnemiopsis embryogenesis. Award funds will help offset the costs required to bring in two project collaborators. My collaborators will transfer embryo manipulation skills vital to the proposed project. These well established and highly successful collaborators will also each give a seminar open to the department during their stay.

Project Goals

The defining characteristic of metazoans is possession of distinct tissues, themselves typically composed from multiple terminally differentiated cell types. However biologists still grapple with understanding the molecular genetic underpinnings of how differential cell fates are established and maintained during an organism's ontogenesis. How is a relatively static genome, shared among all cells within an individual, able to confer the wide range of differentiated cell types we observe?

Among the metazoans, the ctenophores are one of the earliest diverging lineages. Mnemiopsis leidyi, a lobate ctenophore, is poised to become an emerging model system for understanding development in this phylum. Due to the critical phylogenetic position of ctenophores, an improved understanding of their embryonic development is of fundamental importance to identifying developmental events associated with the early evolution of metazoans. In particular ctenophores possess nerve cells, ectodermal and endodermal germ layers, well defined muscle cells, and axes of planar symmetry. Importantly these attributes require a diverse set of differentiated cell fates. Parsimony would suggest that early metazoans possessed a wide range of discrete cell types including multiple defined germ layers and a well developed nervous system.

Developmental and genetic studies are tractable with Mnemiopsis embryos in large part due to their simple cleavage program, optically clear cells, and rapid development. At the eight cell stage Mnemiopsis embryos first acquire an invariant and differential developmental potential resulting in the segregation of specific fates to distinct cell lineages. The four M macromeres uniquely give rise to the nervous system, light producing organs, and the gonads. In contrast the four E micromeres uniquely give rise to the tentacle sheath, the colloblasts, and the modified cilia comprising the characteristic ctene rows.

I hypothesize that a global gene expression analysis characterizing the transcriptomes of the single cell embryo, the E lineage micromeres, and the M lineage macromeres will reveal genes implicated in the initial restrictions of developmental potential in Mnemiopsis. The unique early cleavage program of Mnemiopsis facilitates the use of a forward genetic screen approach aimed at revealing the genetic basis of the earliest restriction of cell fates in a non model system.

For experimental conditions to identify differential developmental potential, collections of E micromeres and M macromeres will be done using embryological manipulations. The eight cell stage occurs at ~85 minutes after egg release and lasts ~15-20 minutes. The E micromeres and M macromeres are easily identified due to their size differences and stereotypic arrangement.
Uncleaved single cell embryos will be dechorionated, then at the eight cell stage, glass needles
dipped in agar will be used to separate blastomeres and mouth pipettes will be used to transfer
separated blastomeres to be flash frozen in aliquots for further processing. One person can do
~100 embryos, thus isolating ~400 E micromeres and ~400 M macromeres/spawn (each embryo
yields 4 E micromeres and 4 M macromeres). With continuously available spawning adults,
multiple spawns/day, and a team of four we can rapidly isolate the large number of blastomeres
required for the experiment.

Career Goals
Research in my lab is focused on investigating patterns of change underlying organismal
diversity. The guiding principle of the research is to understand how specific cell fates are
established and maintained during the course of an organism’s development. *Mnemiopsis* is
particularly well suited to this proposed study due to the unique attributes of it’s early cleavage,
as well as it’s important phylogenetic position relative to other metazoans. There is currently no
better system available for attempting to isolate the full complement of genes that are implicated
in the initial restriction of developmental potential in a non model system.

I expect this novel forward genetic screen on a non model system will reveal a complement
of genes that are involved with the initial segregation of developmental potential during
*Mnemiopsis* embryogenesis. Completion of this project will significantly enhance my career
development. The results from the data will culminate in a significant peer reviewed publication
addressing the molecular genetic basis of the earliest segregation of developmental potential in a
taxa of critical phylogenetic importance. The results will be useful to a broad array of
developmental biologists interested in both early embryonic development and cell fate
determination. Thus I intend on sending this work to a high impact, widely read, journal.
Importantly for my lab, as a forward genetic screen, these comparative RNAseq results will
provide an indispensable reservoir of molecular genetic information that will be used to craft
extramural grant proposals aimed at exploring the functional significance of genes implicated in
cell fate restriction/determination in the early diverging metazoan, *Mnemiopsis leidyi*. I plan to
use the results from this work as an extensive, and core, preliminary data set for hypothesis
testing in a proposal addressing early cell fate determination to be submitted to the
developmental systems cluster of the NSF IOS division.

Budget
I am requesting funds to support the visit of two outside collaborators for one week.

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<th>Amount</th>
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<tr>
<td>Combined estimated airfare</td>
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<tr>
<td>One week hotel @ 150/night x 2</td>
<td>2100.00</td>
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Costs in excess of the $2500 ‘You Choose’ award to support this collaborative skills exchange
and special seminar series will be paid from existing research funds.
BIOGRAPHICAL SKETCH

NAME: Browne, William, E.  POSITION TITLE: Assistant Professor

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>B.A.</td>
<td>1994</td>
<td>Biological Sciences</td>
</tr>
<tr>
<td>University of Chicago, Chicago, IL</td>
<td>Ph.D.</td>
<td>2003</td>
<td>Molecular Genetics &amp; Cell Biology</td>
</tr>
<tr>
<td>Kewalo Marine Lab/University of Hawaii, Honolulu, HI</td>
<td>NSF Postdoctoral Fellow</td>
<td>2003-2006</td>
<td>Developmental &amp; Evolutionary Biology</td>
</tr>
<tr>
<td>Kewalo Marine Lab/University of Hawaii, Honolulu, HI</td>
<td>NAS/NRC Postdoctoral Fellow</td>
<td>2006-2007</td>
<td>Developmental &amp; Evolutionary Biology</td>
</tr>
</tbody>
</table>

Appointments.

a) Positions and Employment

Assistant Professor, Department of Biology/University of Miami.
Assistant Researcher, Kewalo Marine Lab/University of Hawaii.
NAS/NRC Postdoctoral Fellow, Kewalo Marine Lab/University of Hawaii.
NSF Postdoctoral Fellow, Kewalo Marine Lab/University of Hawaii.

b) Honors

INBRE presentation travel award.
Caribbean Coral Reef Ecosystems Program Grant for field research.
FASEB/MARC presentation travel award.
Smithsonian Tropical Research Institute, Postdoctoral Visiting Scientist award.
NASA Life Sciences Grant (PI-Nipam H. Patel, symposium grant).
Prince Fellow, Division of Fishes, Field museum of Natural History, Chicago, IL.

c) Other Experience and Professional Memberships

Member of Society of Integrative and Comparative Biology (SICB)
Member of Society for Developmental Biology (SDB)
Organizing committee, symposium 'The Developmental Basis of Evolutionary Change'; NASA life sciences division (NAG-6033, PI-Patel, NH), University of Chicago.

Selected Publications (10 maximum).


**Synergistic Activities.**

**Training, Mentoring, and Outreach:**
- Undergraduate mentoring via directed research courses.
- Undergraduate; University of Hawaii Honors program support.
- Undergraduate; University of Hawaii MARC-NIH program support.
- Undergraduate student; FURSCA fellowship awardees (2) from Albion College.
- Graduate student; Hanken lab (Harvard) (2004-9), Wimmer Lab (Germany).
- Invited speaker; State of Hawaii, Hanauma Bay Public Educational Lecture series.
- Invited speaker; SCUBAAnauts International, education through exploration series.

**Teaching Curriculum Development:**
- Assist with MBL Embryology Course, Arthropod Module.
- Graduate Core Curriculum course, Manual and Functional Genome Annotation, co-Teach.
- Ecological and Evolutionary Genomics, writing intensive graduate course focused on crafting written synopses and group discussions of field specific primary literature.
- Evolution and Development, writing intensive graduate course focused on crafting written synopses and group discussions of field specific primary literature.
- Integrated seminar/field/lab exercise for University of Hawaii Department of Oceanography graduate course, OCN627 Ecology of Pelagic Marine Animals, R/V Kilo Mo'ana teaching cruises. Course instructor Jeffrey Drazen.

**Material support to broader scientific community:**
- JGI community sequencing project, 'Genomic analysis of amphipod pattern formation'.
- NSF funded Protostome AToL project.

**Reviewer:**
- Funding; NSF, Netherlands Organization for Scientific Research (NWO).
- Journal; Science, Genomics, International Journal of Developmental Biology, ISMEJ.

**v. Collaborators and Other Affiliations.**

a) **Collaborators (previous 48 months):**
- Duman-Scheel, M – Indiana University, School of Medicine
- Dunn, C.W – Brown University
- Gerberding, M – Max-Planck-Institut, Tuebingen, Germany
- Giribet, G – Harvard University, Museum of Comparative Zoology
- Haddock, SHD – Monterey Bay Aquarium Research Institute
- Martindale, MQ – University of Hawaii, Kewalo Marine Lab
- Maxmen, A – Harvard University
- Patel, NH – University of California, Berkeley
- Price, A – Salk Institute
- Schmid, BGM – Georg-August-University, Göttingen, Germany
- Wimmer, EA – Georg-August-University, Göttingen, Germany

b) **Graduate and Postdoctoral Advisors:**
- Graduate Advisor: Patel, NH – University of California, Berkeley
- Postdoctoral Advisor: Martindale, MQ – Kewalo Marine Lab/PBRC, University of Hawaii.
Subject: SEEDS You Choose Award
From: Kathryn Tosney <ktosney@miami.edu>
Date: Thu, 20 Jan 2011 16:42:23 -0500
To: Bill browne <wbrowne@bio.miami.edu>
CC: Natasha Jobbagy Schiller <natasha@bio.miami.edu>

Dear Bill, wbrowne@bio.miami.edu

Congratulations, your SEEDS You Choose application to bring in collaborators for your exciting project has been selected and will be fully funded! Cost share was provided by Biology.

You and the other winners will soon be profiled on the SEEDS home page and on awards page, http://www.as.miami.edu/seeds/. As described in the application, your proposal will be uploaded to the SEEDS site later this term, to form a model for others.

Attached is a SEEDS Quick Guide which will help you when you begin to put your proposal into action. Our SEEDS Program Manager, Natasha Jobbagy Schiller, cc'd here, will help you to process any payments and assist you as needed. Please consult with her prior to making any purchases to ensure that your purchase is within budget and within sponsored programs regulations. All funding should be spent and payments processed by 08/31/12 when our SEEDS grant is scheduled to end.

Please note that you are required to submit a quarterly report with updates on your progress towards achieving your goals outlined in this proposal. A concluding report is also needed, before our SEEDS grant ends.

SEEDS is excited to support your research and we look forward to hearing about your progress.

Best,
Kathryn

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Professor Kathryn Tosney
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view my calendar at http://penguin.bio.miami.edu/calendar/week.php?user_ktosney